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The Bacteriological Determination of Lactose in Blood.

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The difference between the action of *B. coli communis* and *B. paratyphosus* upon lactose, as contrasted with the similarity of the effect of these organisms upon all other common sugars has been known for a long time, and has been utilized by Castellani[1] for showing the presence of lactose in urine. In the work reported in the present communication this fermentation reaction is applied to the demonstration and approximate determination of that sugar in blood.

Plasma was precipitated by the method of Folin and Wu,[2] brought to a slight degree of alkalinity by the addition of a phosphate buffer mixture, and sterilized in a boiling water bath. It was then divided into 2 parts, one of which was inoculated with *B. coli communis,* and the other with *B. paratyphosus A.* Both preparations were incubated for 24 hours at body temperature. They were then again immersed in a boiling water bath, the organisms removed by centrifugalization, and the residual reducing power determined by a method already described.[3] In a great majority of the blood samples taken before breakfast, identical results, within the limits of the colorimetric method, were obtained with the two organisms. Lactose added to blood to give concentrations of 0.1 to 0.005% was completely removed by *B. coli communis* and was not affected by *B. paratyphosus A.* Glucose, levulose, maltose, and galactose added to blood to give concentrations of 0.1% additional sugar were completely destroyed by both organisms. In spite of these experiments it cannot be positively stated that a difference between the reducing power of filtrate inoculated with the two organisms is due to lactose, but it seems very probable that this will be true in a great majority of instances. Because of this uncertainty it seems best to express results in terms of the glucose equivalent of the reducing power. The accuracy of the determinations of the smaller amounts studied was low, as it was not possible to read each final result with a greater accuracy than a glucose equivalent of 0.001%.

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[1] Castellani, A., and Taylor, F. E., *Brit. Med. J.*, 1917, **2**, 853; 1919, **1**, 183; Castellani, A., *J. Am. Med. Assn.*, 1928, **90**, 1773.

[2] Folin, O., and Wu, H., *J. Biol. Chem.*, 1919, **38**, 81.

[3] Hubbard, R. S., and Allison, C. B., Proc. Soc. Exp. Biol. and Med., 1928, **25**, 408; *Clifton Med. Bull.*, 1928, **14**, 35.

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A Biological Method for the Assay of Cortin.

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No satisfactory method for the assay of cortin has been available. The survival and behavior of adrenalectomized cats is unsatisfactory as it requires too much time and too much cortin.

The white rat is more satisfactory because less cortin is required and a large number of animals may be used. The animals which do not need cortin can be ruled out by employing a sufficiently large number. Young rats (50-150 gm. weight) fail to gain weight, or even lose, after removal of both adrenals. The injection of sufficient cortin enables these animals to grow and develop normally. If the cortin is inadequate a majority fail to gain or may even lose within 1 to 3 days. This forms a basis for the test.

From observations on a considerable series of adrenalectomized rats we have developed the method which follows. Male or female white rats, of standard stock, weighing between 75 and 150 gm. are used. Both adrenals are removed through the lumbar path at one operation. They are fed a uniform standard diet and are weighed in the morning at the same time each day. When not being used for assay their growth is maintained by the injection of adequate cortin. Animals allowed to go more than a day or two without adequate cortin must be brought back to normal with cortin before they can be used for assay because more cortin is required to bring them up if they have lapsed. Injections should be made twice daily, preferably 10 to 12 hours apart because some individuals will not grow normally with one injection.

To assay, one uses 3 or more groups containing sufficient numbers to rule out individual variation. A range of doses is employed, one for each group. This makes it possible to determine the minimum dosage which will maintain normal growth. Anything less than this will result in a loss in appetite, a retardation of growth, or even a loss in weight. This becomes apparent within 24 to 72 hours because growing animals are so sensitive. The test can be carried over a period of 3 or more days if necessary. After an assay, the animals used may be quickly brought back to normal weight and growth by giving an adequate amount of cortin. Therefore, the same animals may be used for assay many times.

If rats weighing 75 to 150 gm. are used, it is not necessary to vary the amount of cortin in regard to the weight.

We suggest as a tentative unit for cortin the amount necessary to be injected twice daily for the maintenance of normal growth in an adrenalectomized rat.

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Pathological Lesions Produced in Rabbits Following Intravenous Injection of Concentrated Scarlet Fever Toxin.

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Duval and Hibbard[1] reported in numerous publications the production in rabbits of acute glomerulo-nephritis following the intravenous administration of the bacteria-free toxic principle of *Streptococcus scarlatinae,* which they characterized as endotoxic in nature. Their histopathological material was considered analogous to that observed in human scarlatinal glomerulo-nephritis. Reith, Warfield and Enzer[2] after repeating these experiments concluded that identical renal lesions also occurred in normal rabbits as well as in those injected with suspensions of non-scarlatinal streptococci. They averred that none of the renal lesions produced were typical of human acute glomerulo-nephritis. Rich, Bumstead and Frobisher[3] were able to produce glomerular damage in rabbits by the intravenous injection of bacteria-free filtrates of fresh broth cultures of a virulent strain of *Streptococcus viridans* isolated from the blood in a case of subacute endocarditis with renal involvement. Their histopathological material was typical of acute hemorrhagic glomerulo-nephritis.

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[1] Duval, C. W., and Hibbard, R. J., *J. Exp. Med.*, 1926, **44**, 567; *J. Am. Med. Assn.*, 1926, **87**, 898; *South. M. J.*, 1926, **19**, 858; *New Orleans M. and S. J.*, 1927, **79**, 669; PROC. SOC. EXP. BIOL. AND MED., 1927, **24**, 876; *ibid.*, 1928, **25**, 529; *J. Exp. Med.*, 1927, **46**, 379.

Recent attempts to concentrate scarlet fever toxic filtrates suggested that a more potent toxic principle than that available in the unconcentrated 72 to 96 hours broth cultures might exert a more constant and specific destructive action on the kidneys and possibly be productive of a minimum lethal dose of scarlet fever toxin useful as test material in the standardization of scarlet fever antitoxin. Two years ago, Hartley[4] prepared such a concentrated substance by dialyzing the scarlet fever toxin through collodion bags, in the manner of Walpole.[5] A large part of nitrogen was thus removed and the dialyzed toxin was subsequently precipitated with increasing quantities of normal acetic acid. The precipitate was finally dissolved in normal saline solution. The resultant solution was reduced to one-fortieth of the original filtrate, having a minimum lethal dose for rabbits of "more than 0.2 cc. but less than 0.25 cc." and contained from 0.17 mgm. to 0.21 mgm. of total nitrogen per cubic centimeter. Pulvertaft[6] simultaneously reported the results obtained on chinchilla rabbits by the intravenous injection of scarlet fever toxin concentrated by precipitation with 5 volumes of absolute alcohol. The strength of Pulvertaft's concentrated toxin was more than 10 times that of the original filtrate, contained 90% less of the nitrogenous constituents of the crude toxin and proved fatal in doses varying from 0.5 cc. to 0.1 cc., death usually occurring within 24 hours. By means of this procedure he asserted that the therapeutic efficiency of scarlet fever antitoxins could be estimated inasmuch as complete protection was afforded against the concentrated toxin by the streptococcal antisera.

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[2] Reith, A. L., Warfield, L. M., and Enzer, N., *J. Inf. Dis.*, 1930, **46**, 42.

[3] Rich, A. R., Bumstead, J. H., and Frobisher, M., PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 397.

[4] Hartley, P., *Brit. J. Exp. Pathol.*, 1928, **9**, 259.

[5] Walpole, G. S., *Biochem. J.*, 1915, **9**, 284.

[6] Pulvertaft, R. J. V., *Brit. J. Exp. Pathol.*, 1928, **9**, 276.

More recently Shinn[7] reviewed the claims of Hartley and Pulvertaft and reported that a lethal dose of scarlet fever toxin for rabbits had not been established. Although rabbits were injected intravenously with more than 7 times the dosage of scarlet fever toxin designated by Hartley as the lethal dose for rabbits, Shinn failed to demonstrate a specific lethal action for the toxin and concluded that rabbits were not susceptible for standardization of scarlet fever toxin and antitoxin. Parish and Okell[8] made a rejoinder to Shinn's report by restating their original contention that large rabbits (2 kilo) yielded results analogous to human tests with the Dick toxin and were useful as test animals for estimation of the therapeutic efficiency of antitoxins. Our purpose in this communication was two-fold: firstly, to study the degree of toxicity of the concentrated scarlet fever toxin in chinchilla and the mixed breed of stock rabbits, and secondly, to carefully scrutinize the specific destructive action of the concentrated scarlet fever toxin on the rabbit renal structures.

Experimental: Our 2 lots of toxic filtrates were prepared by incubating Douglas' broth cultures of *Streptococcus scarlatinae,* Dochez S.F-NY.5, for 72 hours at 37° C. Following incubation, the cultures were passed through Berkefeld V candles and the filtrates tested for sterility and total number of skin test doses of toxin per cubic centimeter. Pulvertaft's method of concentrating the crude toxic filtrate by precipitation with 5 volumes of absolute alcohol was carefully followed. Both the filtrate and alcohol were cooled to about 2° C. before being mixed and the whole was allowed to remain in the refrigerator over night, after which the supernatant fluid was decanted. The precipitate was then dissolved in normal saline solution, the final volume being one-tenth that of the original filtrate. The solution was then placed on the water-bath at 37° C. for 1½ hours and frequently shaken. The solution was finally passed through a Berkefeld V candle and according to Pulvertaft's advice was immediately injected intravenously in rabbits in gradually increasing doses. A quantitative estimation was made of the total nitrogen, total solids and total number of skin test doses of toxin contained in each cubic centimeter of the original crude toxic filtrate and the final concentrated product. Table I shows the results obtained with 2 lots of scarlet fever toxin. It is noted that more than 90% of the nitrogenous constituents and total solids of the original crude toxin have been removed and that a 9-fold concentration of the total skin test doses of toxin was attained. Estimation of the latter was made on 3 individuals susceptible to the Dick toxin.

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[7] Shinn, L. F., *J. Inf. Dis.*, 1930, **46**, 77.

[8] Parish, H. J., and Okell, C. C., *J. Pathol. and Bact.*, 1930, **33**, 527.

TABLE I.
Degree of Concentration by Alcoholic Precipitation of *Streptococcus scarlatinae* Toxic Filtrates.

	Original Crude S. F. Toxin (Dochez SF. NY. 5-1) Lot 1	Concentrated S. F. Toxin (Dochez SF. NY. 5-1) Lot 1	Reduction %
Volume, cc.	1,000	100	90
Skin Test Doses per cc.	14,000	125,000	
Total nitrogen per cc.	2.365 mgm.	0.195 mgm.	91.8
Total solids per cc.	36.20 ”	3.08 ”	91.5
	Lot 2	Lot 2	
Volume, cc.	1,000	100	90
Skin Test Doses per cc.	14,000	120,000	
Total nitrogen per cc.	2.350 mgm.	0.193 mgm.	91.8
Total solids per cc.	36.20 ”	2.67 ”	92.6

Degree of Toxicity: Table II shows the results obtained with the intravenous injection in chinchilla and the mixed breed of stock rabbits of doses of unconcentrated and concentrated scarlet fever toxin varying from 0.05 cc. to 10 cc. Although the chinchilla rabbits showed a relatively greater susceptibility to the lethal action of both the unconcentrated and concentrated filtrates, a minimum and specific lethal dose was scarcely discernible either in the chinchilla or in the mixed breed of stock rabbits. The doses causing death varied irregularly between 0.6 cc. and 5 cc. of the concentrated toxin, although one rabbit survived the injection of 5 cc. and another 10 cc. without any apparent toxic effects. The stock rabbits which lived 24 hours after the injection were killed at that time and studied for macroscopic and microscopic lesions. The chinchilla rabbits which survived the first injection were given a second injection 2 weeks later. With one exception these animals died from 12 to 18 hours after the intravenous injection of the concentrated scarlet fever toxin varying from 2 to 5 cc. Autopsies were performed on all animals within a few hours after death. Blood and peritoneal exudate cultures were made on all the animals at time of autopsy and were regularly found to be sterile. No remarkable macroscopic lesions were found in any one animal.

Kidney Histopathology: Although minor pathological differences were observed in individual animals the histopathological picture as a whole was uniform and the differences showed no correlation with

TABLE II.
Lethal action of intravenous injection of unconcentrated and concentrated scarlet fever toxic filtrates in chinchilla (C) and stock (S) rabbits.

Rabbit	Weight kilo.	First injection			Second injection (2 weeks later)		
		Dose, Concentrated S.F. Toxic Filtrate		Result	Dose, Concentrated S.F. Toxic Filtrate		Result
		cc.	Total Nitrogen, mgm.		cc.	Total Nitrogen, mgm.	
C.294	1.9	0.3	0.0822	Survived	4.0	1.096	Died 18 hrs.
C.295	1.8	0.6	0.1644	Died 16 hrs.	—	—	—
C.296	2.5	1.0	0.2740	Survived	5.0	1.370	Died 18 hrs.
C.297	1.9	2.0	0.5480	”	5.0	1.370	” 12 ”
C.298	1.8	5.0	1.370	Died 12 hrs.	—	—	—
C.299	1.9	10.0	2.740	Survived	5.0	1.370	Died 16 hrs.
S.281	2.2	0.05	0.0137	Survived			
S.280	2.0	0.08	0.0219	”			
S.279	2.1	0.1	0.0274	”			
S.278	1.9	0.2	0.0548	”			
S.277	1.9	0.5	0.1370	”			
S.276	1.6	1.0	0.2740	”			
2.275	1.9	2.0	0.5480	Died 18 hrs.			
S.274	2.2	5.0	1.370	Survived			
S.273	1.9	10.0	2.740	Died 12 hrs.			
Unconcentrated Toxin							
C.290	2.4	1.0	2.365	Survived	2.0	0.548	Died 18 hrs.
C.291	2.2	2.0	4.730	Died 16 hrs.	—	—	—
C.292	2.2	3.0	7.095	” 18 ”	—	—	—
C.305	1.9	4.0	9.460	Survived	2.0	0.548	Died 12 hrs.
C.306	2.1	5.0	11.825	”	2.0	0.548	Survived
C.307	2.2	10.0	23.650	Died 12 hrs.	—	—	—
S.287	2.1	1.0	2.365	Survived			
S.286	1.8	2.0	4.730	”			
S.285	2.0	3.0	7.095	”			
S.284	2.1	4.0	9.460	”			
S.283	1.9	5.0	11.825	”			
S.282	2.1	10.0	23.650	”			
S.309	1.9	20.0	47.300	”			
S.310	2.3	30.0	70.950	”			

dosage of the unconcentrated or concentrated filtrates, number of injections or type of rabbit employed. The renal vessels showed a moderate hyperemia, especially of the capillaries and smaller veins. Congestion of the glomerular capillaries was fairly marked in some sections, while only moderately in others. No hemorrhage was observed. The dominant picture was excessive tubular swelling, especially pronounced in the convoluted tubules. The cytoplasm of the latter was markedly granular and the nuclei were only faintly stained. No definite necrosis not attributable to post-mortem changes was seen. The tubular swelling was so severe in several sections that the tubular ends were pushed into the space intervening between the glomerulus and Bowman's capsule. No tubular casts were observed. The glomeruli were in general hyperemic without showing the least trace of hemorrhage. A faint-staining amorphous substance, resembling albuminous exudate, often distended Bowman's capsule. No glomerular adhesions were seen and no cellular reaction of polymorphonuclear or round cell types was found in the interstitial substance, glomeruli or tubules.

Thymus: In several of the chinchilla rabbits the thymus showed similar histopathological changes, consisting in an intense vascular engorgement throughout the thymic substance.

Conclusions: A minimum and specific lethal dose of the concentrated scarlet fever toxic filtrate was not discernible in rabbits, although the chinchilla breed displayed a relatively greater susceptibility to the lethal toxic effects of scarlet fever toxin. The renal changes observed in the rabbit following intravenous injections of unconcentrated and concentrated scarlet fever toxic filtrates were not those of acute hemorrhagic glomerulo-nephritis but rather analogous to lesions observed in milder forms of tubular damage.

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Attempted Production of the Scarlatinal Syndrome with Whole and Filtered Scarlet Fever Faucial Exudate.

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The rôle of *Streptococcus hemolyticus* in scarlet fever is generally admitted to remain as unsettled as the question about the actual cause of this disease.[1] Both Ciuca[2] and Satake[3] failed consistently to produce experimental scarlet fever in a large series of Dick positive human volunteers by swabbing or injecting their tonsils with freshly isolated cultures of scarlet fever streptococci or scarlet fever blood. Specificity of the Dick toxin has not been granted by Bürgers,[4] Cooke,[5] von Gröer and Redich,[6] Friedemann,[7] Meyer,[8] Molkte,[9] Parish and Okell,[10] Smith,[11] Wheeler,[12] Wadsworth,[13] Zlatogoroff and Derkatsch[14] and numerous other investigators. Serological specificity of *Streptococcus scarlatinae* has not been conceded by Bürgers and Wohlfeil,[15] Ciuca,[2] Friedemann,[7] Maclachlan and Mackie,[16] Smith,[11] Wadsworth,[13] Williams,[17] Zlatogoroff and Derkatsch[14] and many others. Failure of the scarlet fever antitoxin to abate septic complications is now generally admitted. This apparent lack of agreement with the group adhering to the streptococcic etiology of scarlet fever recently prompted Zlatogoroff[18] to review the filterable virus theory of the disease. In an extensive series of experiments on rabbits, monkeys and man he claims to have proven that during the incipient stages of scarlet fever a filterable virus is present in the faucial exudate which is capable of activating the otherwise ubiquitous and avirulent hemolytic streptococcus to assume toxigenic and pathogenic properties by which the clinical picture of scarlet fever is produced. When injected either intravenously or subcutaneously in rabbits, monkeys and man the filtered and sterile scarlatinal exudate almost constantly produces the scarlet fever syndrome, with subsequent typical histopathological lesions, as well as changes in the blood-picture, analogous to the epidemic form of the disease. The experimental disease bestows immunity for over 2½ years against reinfection. In rabbits and monkeys, however, the virus alone is capable of producing the disease in the absence of hemolytic streptococci, and yet, upon recovery the animals' blood-serum contains agglutinins for hemolytic streptococci. When suspended in Ringer's or Locke's solutions Zlatogoroff's filterable virus resists dispersed sunlight for 33 days at room temperature and remains active for 35 days when left in the dark. In 0.5% phenol it remains viable for 15 days and heating for 1 hour at 60° C. completely destroys it. The burden of our communication is the results obtained in guinea pigs, rabbits and monkeys *(Macacus rhesus)* with material removed by Zlatogoroff's method from early and moderately severe cases of scarlet fever.

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[14] Zlatogoroff, S. I., and Derkatsch, W. S., *Ztbl. f. Bakt. Ref.*, 1928, **90**, 399 and 507; *J. Inf. Dis.*, 1928, **42**, 56.

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[16] Maclachlan, D. G. S., and Mackie, T., *J. Hyg.*, 1928, **27**, 225.

[17] Williams, A. W., *J. Am. Med. Assn.*, 1929, **93**, 1544; *Am. J. Publ. Health*, 1929, **19**, 1303.

[18] Zlatogoroff, S. I., *Ztbl. f. Bakt. Orig.*, 1929, **113**, 97.

Scarlatinal Material: Exudations in the mouth, fauces and tonsillar crypts were removed on large sterile cotton swabs and immediately immersed in 50 cc. of saline solution contained in Ehrlenmeyer flasks with glass beads. This material was placed in the shaking machine for 2 hours. At that time half of the emulsion was passed through a Berkefeld V candle. Subsequent study revealed that the filtrate was sterile and that the whole faucial exudations contained about 90% of hemolytic streptococci, determined by the poured blood-agar method. Care was taken always to inject the material into the experimental animal within 2 days after removal from the patient. The pH of the filtrate was usually about 5.2-5.8, while the whole exudate varied between 4.8 and 5.2. When left in the refrigerator, the hemolytic streptococci in the whole faucial exudate remained viable about 6 days.

Animal Inoculations: Twelve guinea pigs, 16 rabbits and 30 monkeys were employed in this study. None of the animals reacted positively with 40 skin test doses of the Dick toxin. Previous to the inoculation with scarlatinal material the daily normal curves of temperature, number of leucocytes and differential blood cells were determined for one week in the guinea-pigs and rabbits employed. The absence of natural agglutinins for *Streptococcus scarlatinae* (Dochez N.Y. 5) was likewise determined in the guinea pigs and rabbits. The entire abdomen of all animals were carefully depilated without inflicting abrasions in the skin. Following the injection of the scarlatinal material, each animal was studied daily for changes in physical appearance, while the temperature, number of leucocytes and the differential blood picture were determined in the guinea pigs and rabbits. Except in the monkeys the Dick test was performed weekly with 1 to 40 skin test doses of toxin, while agglutination tests were made one month after inoculation, the antigens employed being *Streptococcus scarlatinae* (Dochez N. Y. 5) and the strain of hemolytic streptococcus isolated from the scarlatinal exudate injected. The following results were obtained: *Guinea pigs* (300-500 gm.): 6 normal guinea pigs injected subcutaneously with 2 cc. of the sterile filtrate of the scarlatinal faucial exudate showed nothing remarkable for one month following inoculation. Six other normal white guinea pigs were injected subcutaneously with a mixture of 0.5 cc. of whole scarlatinal faucial exudate, 0.5 cc. of a 24-hour broth culture of *Streptococcus scarlatinae* (Dochez N. Y. 5) and 1 cc. of melted guinea pig blood-agar reduced to about 50° C. These animals showed nothing unusual during the following month's observation except the occurrence of positive blood cultures of hemolytic streptococci in 3 animals on the fifth, seventh and eighth days respectively, and positive skin reactions (14 to 20 mm. diameters) with 40 skin test doses of the Dick toxin about the fourteenth day after inoculation with the scarlatinal material. Reinoculation of the whole group of animals took place one month after the first inoculation. Three cc. of the whole scarlatinal faucial exudate were injected subcutaneously. Within 24 hours a suspicious erythema appeared in 5 animals. All of these guinea pigs had previously been inoculated with the whole scarlatinal material and 3 had previously given positive skin reactions with 40 skin test doses of the Dick toxin. Definite flaky desquamation took place within a week in these animals. Neutralization of the skin reactions with scarlet fever antitoxin was not completely accomplished. Neither were any remarkable changes observed in the temperature, leucocytic count nor in the differential blood picture during the erythematous phase. Agglutination tests also remained inconclusive. The Dick tests became negative with 40 skin test doses of the toxin about 4 weeks after the second inoculation with the whole scarlatinal material. The erythematous reactions seemed definitely allergic in nature. *Rabbits* (1600-2200 gm.): 3 cc. of the sterile filtrate of the scarlatinal faucial exudate were injected intravenously in 3 white rabbits and subcutaneously in 3 others. Nothing remarkable was observed in these animals during the following 2 weeks. Six white rabbits were injected subcutaneously with 5 cc. of the whole scarlatinal faucial exudate. Within 24 hours 5 of these animals developed a generalized and suggestive scarlatinal rash which led to fine and flaky desquamation during the following week. These animals reacted positively with 40 skin test doses of the Dick toxin about the fifteenth day after inoculation and these reactions became negative 2 weeks later. One month after the appearance of the rash 3 rabbits presented agglutinin-titers of 1-80, 1-160 and 1-320 respectively with the strain of hemolytic streptococcus isolated from the exudate, while no agglutination occurred with the *Streptococcus scarlatinae*

(Dochez N. Y. 5). Reinoculation subcutaneously with 5 cc. of the unfiltered scarlatinal exudate one month after the first inoculation produced a diffuse and mild erythema in 2 rabbits previously injected with the unfiltered scarlatinal exudate. While the Dick test remained negative in all other rabbits these 2 rabbits reacted strongly with 40 skin test doses of the toxin. Neutralization of these reactions with scarlet fever antitoxin was inconclusive. The changes in temperature, number of leucocytes and differential blood picture during the erythematous phase were not remarkable from that observed in the non-reacting animals. The blood cultures remained consistently negative. *Monkeys:* Thirty monkeys *(Macacus rhesus)* were placed at our disposal by the kind permission of Dr. G. W. Corner. Each animal was injected subcutaneously with 4 cc. of the whole scarlatinal faucial exudate. Nothing remarkable was observed in any one animal during one month's observation except local swelling and slight redness at the site of inoculation, which cleared up rapidly without abscess formation.

Conclusions. Sterile filtrates of scarlatinal faucial exudations failed to produce the scarlatinal syndrome in guinea pigs and rabbits. Subcutaneous inoculation of the unfiltered scarlatinal faucial exudations into guinea pigs and rabbits produced irregularly an erythema suggestive of scarlet fever, without any remarkable changes in temperature, leucocytic cell count or differential blood picture. Development of marked skin sensitivity to the Dick toxin following the injection of scarlatinal exudations suggested an allergic basis for the suggestive scarlatinal rash which was followed by desquamation. Monkeys remained entirely refractory to the scarlatinal faucial exudations. A filterable virus in scarlatinal faucial exudations capable of producing the scarlatinal syndrome in guinea pigs and rabbits was not demonstrable.

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Effect of Epinephrine on the Oxygen Consumption of Frogs Before and After Hepatectomy.

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It has been shown that epinephrine injections in physiological doses liberate lactic acid in muscle. From the work of Meyerhof it is known that an increased formation of lactic acid in muscle is followed by increased respiration. The calorigenic action of epinephrine is ascribed to the extra expenditure of energy required in the reconversion of lactic acid to glycogen. This conception raises the following 2 questions: 1. In what tissues is the reconversion of lactic acid taking place? 2. What is the efficiency of the reconversion process? The present experiments deal mainly with the first question.

Frogs were used with the central nervous system destroyed with the exception of the medulla oblongata. Such animals are able to breathe spontaneously but otherwise they are completely immobilized. Oxygen consumption (at 15° C.) was determined by connecting glass vessels, in which the frogs were placed, with Warburg manometers, the CO_2 being absorbed by KOH. Table I shows that the average oxygen consumption during 2 basal hourly periods is of practically the same magnitude. After the second basal hour the frogs were removed from their containers and injected with salt solution in order to test the effect of an injection. A slight increase in O_2 consumption was noted. A subsequent injection of 0.05 to 0.1 mg. of epinephrine into a lymph sack was followed by a marked rise in O_2 consumption, lasting for 2 hours. This result dispels the notion held by some investigators, that the increased O_2 consumption after epinephrine injection is of central origin and is due to the hyperactivity of the animal.

TABLE I.

Influence of epinephrine on O_2 consumption of immobilized frogs.

No. of experiments	cc. O_2 per kilo of frog per hour at 15° C.					% increase in O_2 over average basal	
	Basal			Epinephrine			
	1st hr.	2nd hr.	Saline	1st hr.	2nd hr.	1st hr.	2nd hr.
11; controls	32.8	31.7	35.1	50.2	44.2	51.2	33.1
6; 1st day after hepatectomy	27.7	27.5	30.0	42.9	32.0	51.0	12.7
4; 2nd day after	27.9	28.5	29.6	38.6	32.4	35.0	13.3
3; 3rd day after	25.7	26.3	—	32.8	30.1	26.4	15.8
2; 4th day after	26.9	25.8	—	33.7	29.7	28.2	12.9

The immobilized frogs were hepatectomized. A calorigenic effect of epinephrine, nearly as marked as in frogs with intact liver, was obtained on the first day after hepatectomy. On the second, third and fourth day after removal of the liver the calorigenic effect was less marked and in some individual experiments it disappeared entirely a few hours before death of the animal.

In the frog it has been shown that lactic acid added to the perfusion fluid of muscle is converted to glycogen (Meyerhof *et al.*[1]) and indeed an increased oxygen consumption occurs in the tissues of the hepatectomized frog after epinephrine injection, an effect which is ascribed to the reconversion of lactic acid. In mammals a large part of the lactic acid formed in muscle under the influence of epinephrine is reconverted to glycogen in the liver.[2] Whether or not a calorigenic effect of epinephrine can still be obtained in mammals after removal of the liver has not been decided with certainty; some of the negative results recorded in the literature may have been due to the rapidly approaching end of the animal and not to the removal of the liver. Nor is there definite evidence available as to whether or not blood lactic acid can be converted to glycogen in mammalian muscle.

In frogs kept anaerobically more lactic acid is formed under the influence of epinephrine than in frogs under aerobic conditions.[3] This may be interpreted in the sense that the Pasteur reaction (*i. e.*, the effect of respiration on lactic acid formation) is not particularly disturbed during epinephrine action but a definite conclusion is withheld until the efficiency of the reconversion process has actually been determined.

5178

Low Power Ultraviolet Lamps and the Control of Rickets.

GEORGE H. MAUGHAN AND J. A. DYE.

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In experiments commenced in April, 1929, and recently completed, tests have been made of the antirachitic value of the ultraviolet emitted by tungsten filaments similar to those used in ordinary electric light bulbs. To secure any appreciable amount of therapeutic rays from such sources, bulbs made of the very best ultraviolet transmitting glass must be used. The Corex D bulbs in the lamps employed in these experiments transmit about three-fourths of the vital ultraviolet given off from the filament. Such lamps, at present provided only for experimental purposes, are designated by their producers, the General Electric Company, as CX lamps.

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[1] Meyerhof, O., Lohman, K., and Meyer, R., *Biochem. Z.*, 1925, **157**, 459.

[2] Cori, C. F., and Cori, G. T., *J. Biol. Chem.*, 1928, **79**, 309.

[3] Cori, C. F., and Buchwald, K. W., *J. Biol. Chem.*, 1930, **87**, *Proc.* **38**.

Six experiments included 255 chickens divided into 23 groups. In 2 of the experiments rats were run in parallel series with the chickens.

We first made use of a 500 watt bulb (vitalux glass) on a 110-volt electric circuit at a distance of 5 feet from the floor. Without reflector and at this distance this source was not adequate for satisfactory cure. Some improvement was noticeable but only to the extent of 10 or 15% in 28 days. Control animals were almost cured in this period with 4 exposures of less than 10 minutes each (37½ minutes in all), from a quartz mercury arc.

We have always heated our small brooders by means of electric light bulbs. When exposed to the rays of 60 watt carbon filament lamps, the chickens invariably develop severe rickets. This has also been the case in 2 earlier experiments where ordinary Mazda bulbs were used.

In the second test no sign of rickets appeared in the pen where a 330-hour CX lamp was kept in the brooder. The controls, having a 60-watt carbon filament lamp in the brooder, all developed severe rickets.

In the third and fourth experiments chickens were completely protected against rickets by exposures to CX lamps during the time of feeding. The lamps were placed in aluminum reflectors at such a level and so adjusted as to throw the rays upon the legs of the birds. The distance was in some groups 13 inches and in others 15 inches from the feed pan to the bulb. Table I gives the growth records of these pens and their controls; also a pen where rickets was prevented by exposure to a quartz arc; and also another reared in the sunshine. These growth records are comparable with the data given in x-ray photographs, blood Ca, post mortem examinations, etc.

TABLE I.
Growth Records—Irradiation While Feeding.
Experiment: Prevention of rickets with CX lamp.

	Weight in gm. at end of weeks									
Treatment	1	2	3	4	5	6	7	8	9	10
1000 hr. lamp 110 volts	50	84	140	193	260					
30-volt lamp	53	85	120	190	256	333	428	532	628	754
Quartz Arc 5 min. per day	48	79	126	171	236	308	391	476	577	689
No irradiation	49	81	128	164	203	222	251	291	304	317
Sunshine	47	78	129	171						661

The 110 volt 1,000 hours bulb, was used in a test to determine whether sufficient ultraviolet was present to *cure* rickets. It requires perhaps 4 times as much irradiation for a satisfactory rapid cure as for prevention. Results showed that where the animals were strong enough so that they stood up while eating, this lamp was effective in curing the disease with our set-up. This lamp gives perhaps 2 times the irradiation necessary for prevention. Animals too weak to stand while eating were not much benefited in this experiment. We therefore believe that the irradiation of the food (a mash composed of 57 parts yellow corn meal, 20 parts wheat middlings, 20 parts dried skim milk, 2½ parts ground bone, and ½ part NaCl), did not make it antirachitic. Neither did the rays have much power to penetrate the feathers of the squatting chickens. The 30 volt lamp has about twice as much ultraviolet as the 1,000 hour lamp.

Chickens were so placed beneath a 60 watt 330 hour CX lamp and reflector that their legs only were exposed to the perpendicular rays at 3 inches distance for 20 minutes per day. These animals were cured of the severest type of rickets in 28 days. Recovery was about as rapid as that of animals irradiated 2 minutes daily by the quartz arc at 30 inches distance.

It is probable that the total length of exposure necessary to cure rickets in children is less than in laboratory animals. A reason for this is found in the fact that the animal's body is to a large extent covered by hair or feathers, while that of the child is not. From the results of these experiments we believe that lamps having an intensity equal to those here used have a value in the control of rickets in young infants. Carefully constructed reflectors would perhaps be necessary in order to utilize the radiations emitted to best advantage.

Illinois Section.

Billings Hospital, Chicago, October 21, 1930.

5179

A New Type of Pancreatic Fistula*

LESTER R. DRAGSTEDT, M. LAURENCE MONTGOMERY AND
JAMES C. ELLIS.

From the Department of Surgery of the University of Chicago.

Several types of pancreatic fistulae have been devised for the study of the external secretion of the pancreas since DeGraaf first tied a cannula in the sectioned pancreatic duct of the dog and led it through the skin. This method and in particular the modification introduced by Elman and McCaughan[1] serves very well for acute experiments. The cannula however usually loosens and comes out after 10 to 15 days and the fistula closes or the animal dies. The method of Heidenhain[2] and Pawlow,[3] whereby the piece of duodenal wall containing the entrance of the lower pancreatic duct in the case of the dog is transplanted into the skin of the abdominal wall is more useful for chronic experiments. This has, however, the limitation in that relatively small amounts of secretion are obtained (200 to 300 cc. per day), the excoriation of the skin is very troublesome, the secretion is contaminated by contact with the abdominal wall, and lastly an ascending infection of the pancreas often results via the ducts and the secretion stops almost completely. In an attempt to obviate some of these difficulties the following type of pancreatic fistula was devised.

The duodenum was cut across 2 cm. distal to the pylorus and again about 8 cm. lower down, just below the entrance of the lower pancreatic duct. The pyloric antrum was resected and the lower

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* This work has been conducted under a grant from the Douglas Smith Foundation for Medical Research of the University of Chicago.

[1] Elman, R., and McCaughan, J. M., *J. Exp. Med.*, 1927, **45**, 561.

[2] Heidenhain, R., *Die Bauchspeicheldruse. L. Hermanns Handb. d. Physiol.*, 1883, **5**, 173.

[3] Pawlow, J. P., *Die Arbeit der Verddauungsdrusen.* Wiesbaden 1898.

duodenum anastomosed to the open end of the stomach by the end to side suture. The common bile duct was sectioned near its entrance into the duodenum and implanted either into the stomach or upper jejunum. The upper short section of duodenum containing the entrance of the pancreatic ducts (usually 2 or more in the dog) was converted into a closed sac by inversion and closure of both ends. An external fistula of this duodenal-pancreatic sac was then established by means of a special gold plated cannula, described in a previous publication.[4] This cannula was carefully wrapped with omentum before it was brought through a stab wound in the abdominal wall. Pancreatic juice was collected in rubber bags attached to the cannula. The juice was of course activated by the admixture of the small amount of *succus entericus* secreted by the duodenal sac. Animals have been kept in good condition in the laboratory, by special treatment to be detailed later, for as long as 6 months with no excoriation or digestion of the abdominal wall.

5180

Fatal Effect of the Total Loss of Pancreatic Juice.*

LESTER R. DRAGSTEDT, M. LAURENCE MONTGOMERY, WARREN B. MATTHEWS AND JAMES C. ELLIS.

From the Department of Surgery of The University of Chicago.

While certain of the earlier physiologists, notably Pawlow, had noted various deleterious effects from chronic pancreatic fistula in animals, these were commonly ascribed to the disturbance in digestion resulting from the absence of an important digestive juice in the alimentary tract. Elman and McCaughan[1] were the first to clearly demonstrate that these adverse symptoms were due to the removal of some necessary substance from the body and its excretion in the pancreatic juice. By an improved type of fistula which permitted greater quantities of pancreatic juice to escape than had been obtained by former workers, they found that death resulted in the dog in from 7 to 8 days when pancreatic juice in amounts of from 150 to 400 cc. was lost per day. Confirming these findings

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[4] Dragstedt, L. R., and Ellis, James C., *Am. J. Physiol.*, 1930, **93**, 407.

* This work has been conducted under a grant from the Douglas Smith Foundation for Medical Research of the University of Chicago.

[1] Elman, R., and McCaughan, J. M., *J. Exp. Med.*, 1927, **45**, 561.

Gamble and McIver[2] reported that 2 dogs provided with pancreatic fistulae of the Pawlow type died in 15 to 42 days respectively and concluded that death was due to dehydration and acidosis resulting from the large loss of Na and Cl in the pancreatic secretion. Both Gamble and Elman later found that life could be prolonged by simply restoring the inorganic elements and water that were lost.

The present experiments were in progress when the paper by Elman and McCaughan appeared and they confirm their findings in all essential respects. Animals were provided with pancreatic fistulae of the type described in the preceding paper. They were given 2000 cc. of Ringer's solution intravenously for the first 5 days after operation at which time the fistula was secreting profusely and the dog was in good condition. From 500 to 1500 cc. of pancreatic juice were obtained in 24 hours, the very large amounts being doubtless due to the fact that all of the pancreatic ducts had their outlets into the duodenal sac undisturbed. The intravenous salt solution was then discontinued but the animals permitted food and water *ad libitum.* They promptly became progressively weak and depressed, lost weight markedly and either died or were moribund in 4 to 6 days. The secretion of pancreatic juice continued until death but was considerably reduced in volume. The following changes in the blood chemistry were found: a reduction in the total plasma base from 155 to 130 mM., a decrease in chloride from 101 to 84 mM., a decrease in HCO_3 from 23.0 to 8.3 mM., a shift in the pH from 7.35 to 6.95, and an increase in NPN from 33 to 55 mg., and urea N from 12 to 33 mg. The hemoglobin, red cell count and volume, and blood volume, determined by the dye method, remained within normal limits.

5181

Prolonging Life in High Obstruction by Administration of Salt Solution Below Point of Obstruction.

HILGER P. JENKINS. (Introduced by L. R. Dragstedt.)

From the Department of Surgery, the University of Chicago.

The cause of death in acute intestinal obstruction has been most generally attributed to a toxaemia. The nature of this toxaemia has not been definitely established. Although the toxic theory has been used to explain death in all types of obstruction, recently some investigators have pointed ont that there is a difference between simple high obstruction in which vomiting is a conspicuous feature and obstruction with injury to the blood supply by distension or strangulation. They believe that the latter results in a toxemia; however the rapid death that occurs in the former they ascribe to loss of digestive secretions alone.

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[2] Gamble, J. L., and McIver, M. A., *J. Exp. Med.*, 1928, **48**, 859.

To determine the effect of salt solution administered into the bowel below the point of an uncomplicated high obstruction, dogs were obstructed from 3 to 24 inches beyond the duodeno-jejunal junction and the stump of bowel below the obstruction brought to the outside as a jejunostomy. Through this the animals were given daily a total of 1000 cc. of water with 50 gm. of glucose and 10 gm. of sodium chloride. This was divided into 10 feedings, at hour and a half intervals.

In a series of 19 animals most of them died during the first week from a complication such as pneumonia, peritonitis, or from pulling the bowel out through the jejunostomy wound. Five survived from 13 to 26 days but died from complications. One animal lived 52 days. The behavior of this animal throughout the period of obstruction was remarkable. He was lively and would run about like a normal dog until shortly before death. Vomiting occurred about every other day. On several occasions a watery diarrhea developed when more than 5% glucose was used. The weight gradually declined from 14.2 to 6.4 kg. The blood chemistry taken weekly showed a slight rise in chlorides from 472 to 520 during the first 6 weeks. On the forty-sixth day it was 565 and on the fifty-second day it was as high as 840. The CO_2 showed slight variation and was practically the same on the forty-sixth day as it was before operation. The N.P.N. fluctuated from 40 before operation to 53 on the fifty-second day. In the other animals which survived a week or longer the blood chemistry showed no tendency to fall in chlorides but rather a rise which in some instances was quite marked. At autopsy the animal which lived 52 days showed no evidence of a complication which could explain death other than the long standing obstruction and emaciation. The obstructed bowel measured 95 cm. from the pylorus. It was distended especially above the point of obstruction. The wall of the distal 15 cm. was several times thicker than the bowel higher up which was equally distended. The bowel from the jejunostomy down was collapsed.

White and Fender[1] in a similar experiment kept an animal alive with high obstruction for a month by introducing the vomitus along with glucose and water into a jejunostomy. Several investigators have been able to keep dogs alive with high obstruction as long as 4 weeks by the daily injection subcutaneously of salt solution. The total loss of gastric juice and of pancreatic juice is rapidly fatal. When sodium chloride or Ringer's solution is administered to these dogs life is prolonged.

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[1] White and Fender, *Arch. Surg.*, 1930, **20**, 897.

When digestive secretions are lost by vomiting in obstruction or by fistula experiments animals will rapidly die. When the secretions are replaced by salt solution the animals will survive for a considerable length of time. It appears very unlikely that salt solution introduced into the bowel would prevent any toxaemia that might occur from the obstruction.

5182

Studies in Capillary Fragility.

IRVING S. CUTTER and GILBERT H. MARQUARDT.
(Introduced by A. C. Ivy.)

From the Department of Medicine, Northwestern University Medical School.

One of us (Irving S. Cutter) has been interested for many years in the production of blood extravasations such as petechiae by trauma. We are all aware that the amount of force or injury necessary to produce such hemorrhagic extravasations varies greatly in different individuals. Investigation of such a problem naturally led us to a study of the blood vessels of the skin and specifically of the capillaries.

Using a Muller Weiss skin microscope (Zeiss) we first familiarized ourselves with the technic of capillary observations. The end of the nail bed is by far the most satisfactory location for observation. This, because there is a single row of capillary loops extending towards the finger tip, with no confusing venous network underlying. In this area one is able to follow the arterial and venous sides of the capillary loops for quite a distance and usually to see the blood flow.

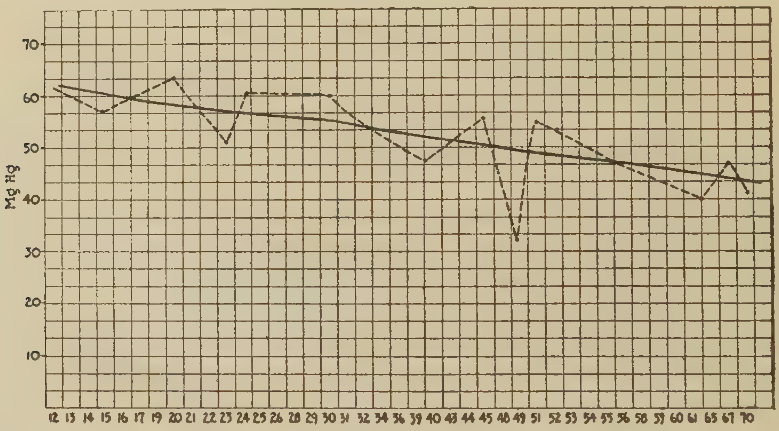
It was our intention to produce ruptures of the capillary walls by means of measured negative pressure—thus measuring the capillary fragility.

This report and accompanying graph are based on 200 measured cases.

Method of Experimentation. The fourth finger of the left hand was used in all cases with the patient sitting and the hand on a level with the heart. The nail bed was lightly cleansed with a 3 to 5% salicylic acid solution, and the finger then inserted into a glass finger holder which is attached to the negative pressure apparatus. The column of mercury is then slowly raised. The finger is removed every few millimeters of mercury to look for signs of capillary rupture.

About half the cases show the rupture point by petechiae. In the other half one must examine the capillaries, and assume that a gap in the capillary line (not previously noted) means that rupture has occurred. We were unable to produce capillary damage in 14 cases.

The accompanying graph is the record of 200 consecutive cases, grouped according to ages.



Our results show definitely that fragility increases in direct proportion to age increase. The exceptions, such as the 49-year-old group, are produced by cases having marked generalized pathology. (The average blood pressure in this group is 280—systolic.) Another interesting observation is that ruptures were produced by the lowest pressures in cases which had systolic pressures above 200 millimeters mercury. The data are now too meager to make a comparative statement where the systolic pressure is above 150 mm. of mercury. We are also led to believe, from our observations, that all chronic diseases such as rheumatic heart disease, diabetes, etc., predispose to increased capillary fragility.

There is as yet no accurate method of measuring sclerosis. The internist palpates the brachial artery or the ophthalmologist looks at the fundus of the eye and an inaccurate, understandardized opinion is given in most cases. Localized sclerosis is known to occur at the base of the brain, in the circle of Willis and in certain organs. The method would be of no value in such cases, but sclerosis is more often a generalized than a localized picture. We hope that this method may prove to be of some use in determining the likelihood of sclerosed vessels, and the degree to which vessels are sclerosed.

The interposition of thick horny skin makes observations very difficult. Negroes are of no value because of a pigment refraction.

While many of the above observations have been repeated on the same individual we are not entirely satisfied with the method and shall try to improve it. The final report on the clinical efficiency of this method should include hundreds and perhaps thousands of measurements. In our next report we shall classify measurements according to age—blood pressure and disease groups.

If we had omitted our known pathologic cases in making the graph, a more uniform curve would have resulted showing the correlation between age and fragility of the capillaries.

5183

Effect of Some Imidazoles on Gastric Secretion.

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From the Department of Physiology and Pharmacology, Northwestern University Medical School.

Histamine and pilocarpine are imidazoles which are known to stimulate gastric secretion.

Since gastrin preparations give a strong Pauly reaction and produce a number of the pharmacological effects of histamine, the question of the identity of histamine and gastrin has been raised[1] although some differences in the chemical properties of gastrin preparations and histamine are known to exist.[2] Koch, Luckhardt and Keeton[2] concluded from their work that gastrin appears to be a basic imidazole derivative. They studied the effect of 3 imidazole derivatives, namely, histidine, methylimidazole HCl and hydroxymethylimidazole HCl, on gastric secretion with negative results.

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[1] Ivy, *Physiol. Reviews*, 1930, **10**, 282.

[2] Koch, Luckhardt and Keeton, *Am. J. Physiol.*, 1920, **52**, 508.

Ivy and Javois[3] found also that histidine subcutaneously did not stimulate gastric secretion. Because of these facts we thought that it would be worth while to study the effect of other imidazoles on gastric secretion.

Professor Rose of the University of Illinois supplied us with imidazole proprionic acid, imidazole aldehyde, d-imidazole lactic acid and imidazole acrylic acid. Professor Hurd of Northwestern University supplied us with imidazole.

These imidazole derivatives were injected subcutaneously into 3 Pavlov pouch dogs a number of times in doses of from one to 5 mg. with negative results. Larger amounts were not used because Burgess, Sacks and Ivy[4] have had gastrin preparations which were active in less than 0.5 mgm. doses.

These 5 imidazoles were also injected intravenously in dogs prepared for recording blood pressure changes. None of them in from one to 5 mg. doses had any effect on blood pressure.

It is interesting that β-imidazole ethylamine HCl is a very potent excitant of gastric secretion, whereas imidazole and the ethylamine HCl[3] are inactive.

Conclusion. Imidazole, imidazole proprionic acid, imidazole aldehyde, l-imidazole lactic acid, and imidazole acrylic acid in doses of from one to 5 mg. have no effect on gastric secretion or blood pressure.

5184

Experimental Hepatogenous Cholecystitis.

EDMUND ANDREWS AND LEO HRDINA.

From the Department of Surgery of the University of Chicago.

Gall bladder bile in dogs cultured in our laboratory has been shown by Rewbridge[1] to be almost invariably sterile.

In a series of experiments in which biliary stasis had been present for varying periods of time *B. welchii* and lesser numbers of cocci and *B. coli* were grown from the bile in 76% of cases. Biliary stasis had been produced not only by ligating the cystic duct, care being taken to avoid the cystic artery, but also by common duct ligation and by both procedures.

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[3] Ivy and Javois, *Am. J. Physiol.*, 1925, **71**, 583, 591, 604.

[4] Burgess, Sacks and Ivy, unpublished data.

[1] Rewbridge, Allan G., and Hrdina, Leo, PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 528.

In most of these experiments not only were these bacteria present but marked evidences of cholecystitis were found. Gross specimens of these gall bladders revealed the interesting fact that the infection undoubtedly made its way into the gall bladder not through the bile or the blood stream but by direct extension from the liver. This could be seen by killing the dogs in various stages of the process. In the early stages (1-4 days), the gall bladders presented the picture of a cholecystitis only on the hepatic surfaces. The free peritoneal side at this period is thin, normal in appearance both grossly and histologically. The hepatic surface is thickened, often to 10 times normal and its wall shows edema and infiltration of leucocytes and round cells. Later the inflammation extends around the entire circumference of the viscus. The mucosa generally remains quite normal even in the later stages when the other layers of the wall show marked inflammation.

The work of Dragstedt[2] and later of Andrews[3] on the rich anaerobic flora of the liver has shown that these organisms are constantly present and it now becomes evident that in stasis they may make their way into the gall bladder in large numbers.

The recent clinical experience[4] of finding *B. welchii* in many cases of fulminant cholecystitis makes it seem quite possible that this route of infection may be a common one in the human being.

5185

Ovulation in the Rabbit as a Diagnostic Measure in Early Pregnancy.

P. F. SCHNEIDER. (Introduced by C. A. Dragstedt.)

From the Department of Physiology, Northwestern University.

In a previous communication,[1] a preliminary report of 100 cases has been submitted, demonstrating the production of ovulation in immature female rabbits by the intravenous injection of urine from pregnant women, and its application as a diagnostic measure in early pregnancy. The work of Friedman[2] on ovulation in the rabbit indicated that the principles of the Aschheim-Zondek[3] test for pregnancy might be applied with greater facility by the use of rabbits instead of mice. Of the 100 cases previously reported, 50 were used as controls, the urine being obtained from definitely known pregnant and non-pregnant patients; and in the remaining 50, urine was obtained from women in whom pregnancy was suspected but could not be diagnosed by the usual methods. This was done to determine the value of the test in the diagnosis of early pregnancy, and to attempt to outline its limitations. The results demonstrated a degree of accuracy comparable to that obtainable by the Aschheim-Zondek test, indicating possibilities which stimulated further investigation along these lines. Since that time an additional series of 100 tests have been carried out, and a brief report of the combined result is made.

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[2] Ellis, J. C., and Dragstedt, L. R., *Arch. Surg.*, 1930, **20**, 8.

[3] Andrews, E., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 987.

[4] Gordon-Taylor, G., and Whitby, L. E., *Brit. J. Surg.*, 1930, **17**, 78.

[1] Schneider, *Surg., Gynec. and Obst.*, in press.

One female rabbit, 16 weeks of age, is used for each test. From 5 to 7 cc. of a voided first morning specimen of urine is injected into the marginal ear vein. Autopsy is done 30 hours after injection and an immediate diagnosis is possible by macroscopic inspection of the ovaries, depending on the presence or absence of corpora hemorrhagica and corpora lutea. The description of this technique, when compared with a description of the Aschheim-Zondek test, emphasizes its simplicity and practicability.

In the complete series of 200 tests, 50 were carried out as controls, while 150 were used as actual test cases in which pregnancy was suspected but could not be diagnosed by the usual methods. In 25 of the control cases the specimen was obtained from patients late in the pregnancy where a positive diagnosis could be made by other methods. The remaining 25 specimens were obtained from patients who were known not to be pregnant. The known pregnancies all resulted in the occurrence of ovulation in the rabbit, while the negative controls all gave negative results.

Of the 150 test cases there were 61 positive and 89 negative results obtained. Of the positive series, subsequent events proved the test to be correct in all except 2 instances. It appears on repetition of the test that these discrepancies can be accounted for only by an error in the markings of the rabbit in one instance, and improper interpretation of the macroscopic findings in the other instance. Both tests yielded negative results when repeated using the same specimen, and were merely technical errors subject to correction. Of the

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[2] Friedman, *Am. J. Physiol.*, 1929, **90**, 617; **89**, 438.

[3] Aschheim, *Am. J. Obs. and Gyn.*, 1930, **19**, 335.

89 negative cases there have been 6 instances in which repetition of the test at later dates gave positive results. This was due to the fact that in these 6 instances, the urine was obtained so early in the pregnancy that the concentration of the hormone in the urine was insufficient to cause a positive reaction, and therefore due to limitations of the test which future investigation may overcome.

The data obtained in these cases indicates that the reaction appears at approximately 18 to 21 days following the intercourse responsible for the pregnancy. If these observations are proved to be correct by subsequent investigation, they will serve as additional evidence to substantiate the theory[4, 5] that the hormone causing the reaction may be dependent on the development of the decidual tissue instead of entirely an anterior pituitary product as was at first maintained.[6] Further evidence supporting this contention is derived from the fact that the only conditions other than pregnancy causing the reaction are hydatidiform mole and chorio-epithelioma. There has been one case in this series of chorio-epithelioma following hydatid mole, which was accompanied by a positive reaction up to one week following complete hysterectomy, and a negative reaction 5 weeks after operation.

Conclusion. The method provides a positive test for pregnancy after the third week, which can be completed in 30 hours, and due to its simplicity does not require extensive laboratory equipment. The factors involved indicate the possibility that by a rapid method of extracting the hormone from the urine and injection of a concentrate of the hormone, the scope of the test may be increased by reducing the time element. There is also the possibility that injection of the hormone in concentrated form would produce positive results at an earlier stage of the pregnancy than is now possible.

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[4] Collip, *Canad. Med. Assn. J.*, 1930, **22**, 761.

[5] Zondek, *Endokrinol.*, 1929, **5**, 425.

[6] Aschheim and Zondek, *Arch. Gynakol.*, 1927, **130**, 1.

5186

Blood Chloride Changes in Relation to Diuresis in Dogs.

H. L. HANSEN, L. S. FOSDICK AND C. A. DRAGSTEDT.

From the Department of Chemistry of the Dental School and the Department of Physiology and Pharmacology, Northwestern University.

There is considerable practical as well as theoretical importance to a complete explanation of the mode of action of the various types of diuretics. In various recent contributions to this field, Curtis has studied the problem in rabbits and upholds the view that the specific diuretics have their primary action on the tissues, mobilizing electrolytes, principally chlorides, which in turn act as stimulants to the kidney and increase the formation of urine. Various workers have pointed out certain apparent differences of behavior between the rabbit's kidney on the one hand and that of dog and man on the other. It seemed advisable, therefore, to determine the effect of certain diuretics on the blood chloride levels in relation to the diuretic response in dogs.

Female dogs were prepared for urine collection by transplanting the trigone of the bladder to the anterior abdominal wall. This procedure obviates the effects of catheterization and enables accurate observations on urine flow to be made. Blood was drawn from either the saphenous or external jugular vein and the chlorides determined by the micro method of Van Slyke. These determinations were checked in many instances by Van Slyke's macro method. Diuresis was produced with euphyllin (theophylline ethylene diamine), salyrgan, and novasurol. No consistent significant variations in the blood chloride level were noted either preceding, during, or following the diuresis. Such negative results might be due to the activity of the kidney so that any tendency to a rise in the blood chloride level might be offset by increased excretion. To test this possibility several dogs were nephrectomized. Twenty-four hours later, these same diuretics were administered and the blood chloride level followed. No significant changes were observed. Another method of testing the ability of these diuretics to mobilize chlorides from the tissues to the blood stream was tried. Distilled water was injected into the peritoneal cavity of dogs. The injected fluid tends quickly to become isotonic and concurrently the level of the blood chloride tends to fall. If chlorides are mobilized from the tissues to the blood stream by the diuretics it would be expected that under their influence there would be a lessened fall in the blood chloride level particularly if the kidneys were removed. Such an experiment was performed. In 3 dogs, nephrectomized 24 hours previously, the intraperitoneal injection of 300 cc. of distilled water caused a drop in the blood chloride levels of 20, 29, and 23 mg., respectively. In 2 similar experiments when salyrgan was administered simultaneously with the distilled water the drop in the blood chloride level was 0 and 14 mg., respectively and in one experiment with euphyllin the drop was 9 mg. These differences, although small, are definitely greater than the errors of the methods of determination and may possibly be interpreted as evidence of an extrarenal action of these diuretics that would tend to aid or augment their diuretic action. In view of the results in the other experiments and of the available data in the literature, we feel that such an extrarenal action is probably of minor importance compared with the action on the kidney insofar as diuresis in the dog is concerned.

5187

Rate of Heart-Beat in Limulus as Affected by Exposure to Ultraviolet Point Radiation.*

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From the Department of Physiology, University of Chicago, and the Department of Biochemistry, Loyola University Medical School.

This is the third of a series of studies on heart-beat. Preliminary reports have appeared in this journal, covering experiments with embryos and newly hatched Fundulus, and with adult frogs and turtles.[1, 2] It has been possible, in each instance, to modify the rate of beat by exposure to ultraviolet radiation, using the unscreened spectrum of a Cooper-Hewitt quartz mercury vapor arc running at 110 volts, D. C., focussed through a quartz rod.

The present report deals with experiments on the Limulus heart, the beat of which is controlled by a nerve cord which runs along the median dorsal surface of the heart.[3] Exposures were made by placing the tip of the quartz rod on various points along the cord. In this way, an increase or decrease in rate of beat was obtained, with the length of the period of exposures and the locus of application of the rod as determining factors. In general, an increase in rate of beat was obtained by short exposures of a given region, and a decrease in rate following longer exposures. The rate of beat could also be slowed by placing the rod on the nerve cord at points of entrance of inhibiting nerves. Rapid recovery of normal rate followed exposures in which the induced variations were not marked.

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* Supported in part by a grant to the University of Chicago from the Rockefeller Foundation, and in part, by a grant from the Radiation Fund of the National Research Council.

[1] Hinrichs, M. A., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 354.

[2] Hinrichs, M. A., and Johnson, P. O. C., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 971.

[3] Carlson, A. J., *Am. J. Physiol.*, **12**, 67.

It was early noted that any pressure on the nerve cord due to too heavy a contact of the rod had to be avoided since it slowed the rate of beat or even blocked conduction.

In many experiments where the normal rate of beat was studied for a considerable period of time, there were distinct indications of a rhythm consisting of a series of slightly faster beats alternating with a series of slower ones. Short exposures did not abolish this rhythm, although it increased the rate of beat throughout.

Cutting transversely through the heart muscle did not eliminate the synchronism of the beat in the two parts of the heart, nor did it affect the conducting power of the nerve cord during exposure.

No change in rate of beat of the entire heart could be demonstrated by direct exposure of the heart muscle itself, nor could the beat be influenced by exposure of the suspensory ligaments. Immediately local contractions of muscular tissue followed exposure of isolated ganglion cells situated in the muscle tissue.

5188

Effect of Estrin Injections on Reproductive Organs, Hypophysis, Kidney, Adrenals, Thyroid and Blood Vascular System.*

M. M. KUNDE, F. E. D'AMOUR, R. G. GUSTAVSON AND A. J. CARLSON.

From the Hull Physiological Laboratory and the Department of Physiological Chemistry of the University of Chicago.

Female sex hormone obtained from human pregnancy urine, prepared and assayed as previously described (D'Amour and Gustavson[1]) was daily injected into 4 immature dogs (3 females and 1 male) over a period of time 6 to 17 weeks in duration. The daily doses varied from 25 to 800 rat units. At the end of 4 months histological examination of the dog receiving the largest dosage revealed essentially no pathology in the heart, arteries, liver, spleen, adrenals and thymus. The tubules of the kidneys contained some albumin; the glomeruli were normal. Hyperplasia of the thyroid was marked. Maxillary prognathism in these dogs was demonstrable. The anterior lobe of the hypophysis was much smaller than in the normal dog. The cytoplasm of the chromophobes of this lobe was diminished in amount. The posterior lobe seemed relatively much larger than in the normal dog. The nipples of all dogs enlarged. The external genitalia and uterus of the female dogs *increased* in size. Hyperplasia of the glands of the uterine endometrium was pronounced but no hemorrhagic discharge was observed. The ovaries of the experimental dogs were less than half the size of the control dogs, containing relatively more stroma and no mature follicles.

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* This investigation was aided in part by grants to the University of Chicago from the Rockefeller Foundation and the National Research Council.

[1] D'Amour, F. E., and Gustavson, R. G., *J. Exp. Pharm. and Therap.*, 1930.

5189

An Egg White Digest Medium for the Gonococcus.*

C. PHILLIP MILLER, JR., AND RUTH CASTLES.

From the Department of Medicine of the University of Chicago.

The culture medium described below has been found to yield a more plentiful growth of gonococcus than any we have employed. Additional advantages are transparency, low content of protein, and ease of preparation.

Dissolve 40 gm. of powdered egg white† in a liter of 0.45% NaCl solution and adjust the reaction to pH = 7.0-7.4. Rub into a paste and add to the solution 4 gm. of a special high-test trypsin;‡ plug the mouth of the flask with cotton and incubate on a water bath at 48° C.—with occasional agitation—for 10-12 hours. This temperature prevents spoilage by inhibiting the growth of most micro-

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* This study was made possible by a research grant from the Public Health Institute of Chicago.

† We have used both Merck's and Mallinckrodt's preparations of egg white and found them equally satisfactory. They are purchased under the label: "Albumin egg, impalpable, powdered."

‡ This trypsin, which we found superior to the others we have tried, was kindly furnished us by Dr. David Klein of the Wilson Laboratories, Chicago.

organisms. By the end of 10-12 hours, a sample of the digest should give an amino acid titration of 130-180 (*i. e.,* cc. of N/10 NaOH per 100 cc. of digest) by Sörensen's method. This determination is not essential, except as proof that digestion has proceeded satisfactorily, for we have found that a digest with a Sörensen titer of 80 supported growth as effectively as one with a titer of 180.

Now add sufficient HCl to bring the reaction down to pH = 5.0, the isoelectric point of most of the proteins, and autoclave for 15 minutes. Add sufficient distilled water to bring the volume up to its original quantity. Then separate the precipitated proteins by filtration through paper or by centrifugation, and then by filtration through a Berkefeld candle. This sterile filtrate can be stored in the ice-box until needed.

To this clear, straw-colored filtrate add 6 cc. of 0.04% phenol red solution per 100 cc. and readjust the reaction to pH = 7.0-7.2 with NaOH. The indicator in this concentration exerts no bacteriostatic action on the gonococcus, and its addition directly to the digest simplifies the adjustment of pH. It may be necessary to withdraw samples of the fluid in order to determine its reaction in a comparator block, but with a very little experience one is able to estimate from the color of the large bulk of fluid its pH accurately enough for the purpose at hand.

To make a liter of medium put together:

250.0	cc.	digest
750.0	"	distilled water
0.2	gm.	KCl
3.0	"	NaCl
1.25	"	$NaHCO_3$
10.	"	dextrose
15-20.	"	agar depending on the consistency desired.

Heat and stir the mixture until all of the ingredients are dissolved. If necessary, adjust the reaction to about pH = 7.2-7.4. A simple and sufficiently accurate method of making readings is to drop about a cubic centimeter of the hot solution on to a piece of clean white paper and note the color of the cooled agar. Then add: 8 cc. of a M/15 solution of Na_2HPO_4 and 2 cc. of a M/15 solution of NaH_2PO_4.

The medium is now ready to be tubed, autoclaved, and slanted. Freshly slanted medium yields a better growth than that which has dried out even a little. The final reaction should be pH = 7.0-7.4.

The digest prepared according to the directions given contains no heat-coagulable proteins and gives a negative phosphotungstic acid test. Half saturation with ammonium sulphate yields a precipitate which gives a positive biuret test. On full saturation of the filtrate a precipitate is obtained which also gives a biuret test. It therefore contains both proteoses and peptones.

If the presence of undigested proteins in the medium is a matter of indifference to any one using it—that is, if their possible adherence to organisms removed from the culture does not interfere with the use to which the organisms are put—the acidification and autoclaving of the digest can be omitted. The medium in that case is rendered cloudy on sterilization by the precipitation of the heat-coagulable proteins.

Another step which can be omitted is the filtration of the digest through a Berkefeld candle. Its purpose is to render the digest sterile—in order that it may be stored with safety—and perfectly clear, for occasionally a very fine precipitate forms on autoclaving which passes a paper filter and makes the medium cloudy.

The only fault we have found with this medium is that cultures of gonococcus do not remain viable longer than about 12 hours. They develop very rapidly within that time but die shortly afterward. This condition obtains for both freshly isolated and stock strains.

5190

Transplantation of Ureteral Segments to the Abdominal Wall.

C. B. HUGGINS. (Introduced by L. R. Dragstedt.)

From the Department of Surgery of the University of Chicago.

In previous communications[1, 2] an osteogenic effect of the epithelium of the urinary bladder, ureter and renal pelvis was demonstrated when the epithelium was brought into association with certain connective tissues such as the muscles and fasciae of the abdominal wall, extremities, etc., in the dog.

These observations on the ureter have been repeated and extended. In 4 dogs a 3 cm. segment of the ureter was excised and split along its long axis. This rectangular piece was then sutured on the right internal oblique muscle with silk and the wound closed. The experiments were terminated at 35, 42, 62, and 79 days and in all cases an epithelial lined cyst was found partly surrounded by bone.

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[1] Huggins, PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 349.

[2] Huggins, *Arch. of Surg.*, in press.

In the same 4 dogs in a corresponding part of the left internal oblique muscle a 3 cm. segment of the ureter, not split but left in its original tube form was sutured. The ends were not ligated. Examination 35, 42, 62 and 79 days later showed (1) the ends of the transplant had closed, (2) the lumen of the transplant contained a small quantity (0.5-1.0 ccm.) of cloudy fluid, (3) bone had not formed around the transplant.

Histological examination showed the transplant lined with transitional epithelium; a rather large amount of smooth muscle of the ureteral wall has survived the transplantation.

5191

Effect of Calcium Chloride Injections on the Blood Sugar of Normal and Jaundiced Dogs.*

ALLAN G. REWBRIDGE AND EDMUND ANDREWS.

From the Department of Surgery of the University of Chicago.

The complicated interrelationship between blood coagulability and its content of calcium and sugar has been the field for much study. Therapy of jaundice by the administration of calcium was popularized by the publications of Walters.[1] Attempts to give these procedures a scientific basis have not always been successful. However, the observations of many clinicians have shown beyond doubt that intravenous injections of calcium chloride are of definite value. More recently the older views have been revived and glucose both by feeding and by injection has become a favorite method of therapy. The experiments of Ravdin[2] have established the fact that raising the sugar level in the blood is of far more importance in increasing the coagulability than raising the calcium content and this procedure has many enthusiastic advocates.

Wright and Cowan[3] in a recent communication have established the fact that injection of sugar in both normal and jaundiced dogs produces a significant rise in the blood calcium and that this rise persists after the blood sugar has returned to normal levels and that the coagulability of the blood parallels the calcium level and not the sugar curve. With these points in view it was decided to ascertain the effect on the blood sugar level of calcium injection and as can be seen from the accompanying table the rise in blood sugar after calcium injection is nearly as marked as after injecting sugar itself and tends to last longer. The average rise in blood sugar is 62 mg. per 100 cc. or about 70%.

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* This work was done in part on a grant from the Douglas Smith Foundation of the University of Chicago.

[1] Walters, W., *Minnesota Med.*, 1925, **6**, 203.

[2] Ravdin, I. S., *et al.*, *Annals Surg.*, 1930, **91**, 801.

[3] Wright, H. N., and Cowan, D. W., loc. cit., 1930, **27**, 950.

TABLE I.
Effect of $CaCl_2$ injection of blood sugar.

Time hrs.	Jaundiced Dogs										Normals		
	1	2	3	4	5	6	7	8	9	10	[illegible]		
0	92	103	93	84	101	89	75	99	103	184	122	105	125
½	109	117		104	123	92	136	118	119	149	170	127	145
1	105	111		119	134	102	155	156	158	200	160	136	138
1½	122	120	112	145	148	107	181	176	178	184	142	123	128
2	134	124	170	143	189	115	187	178	172	238	175	132	175
2½	149	110		163	198	108	176	167	167	280	156	120	155
3	120	101		151	191	103	183	164	200	280	193	118	125
4	122	111	156	129	150	187					140	140	140
5		110	92								126	108	137
6		92	92	98	107	95							
8	99												
24		100				89							

1. Cholecystectomy and common duct ligation 7 days P. O. Wt. 12.5 k. 10 cc. 10% $CaCl_2$
2. Same dog. 9 days P. O. 15 cc. 10% $CaCl_2$.
3. Same procedure. 8 days P. O. Wt. 15 K. 15 cc. 10% $CaCl_2$.
4. Same 7 days P. O. 10 cc. 10% $CaCl_2$.
5. Same 10 cc. 10% $CaCl_2$.
6. Same 8 days P. O. 15 cc. 10% $CaCl_2$.
7. Common duct ligation and right nephrectomy. 7 days P. O. 10 cc. 10% $CaCl_2$.
8. Same.
9. Common duct ligation and double nephrectomy. 2 days P. O. 10 cc. 10% $CaCl_2$.
10. Same.

Normals. 10 cc. 10% $CaCl_2$.

This fact brings us back to where we were before the paper of Wright and Cowan was published. The 2 factors, sugar and calcium, seem to have a reciprocal influence on each other and it is therefore impossible to say which one is of the greater importance in the control of blood coagulability.

5192

An Experimental Study of Fat Necrosis in Bile Peritonitis.*

ALLAN G. REWBRIDGE. (Introduced by E. Andrews.)

From the Department of Surgery, University of Chicago.

During a recent study of the reaction of the peritoneum to sterile bile, fat necrosis was frequently observed in the mesentery near the pancreas. The term fat necrosis has come to mean a special form of necrosis characterized by the hydrolysis of neutral fat into fatty acids and glycerol, the former combining with calcium to form insoluble soaps.

In a series of 20 dogs after ligating the common bile duct and making a stoma in the gall bladder, bile was allowed to drain into the peritoneal cavity. Eighteen hours later the peritoneal cavity presented the picture of an acute severe general peritonitis and contained several hundred cubic centimeters of a serosanguinous exudate in which were observed polymorphonuclear leukocytes and *B. welchii.* Areas of fat necrosis 1 cm. in diameter were present in the fat of the mesentery adjacent to the pancreas in 15 out of 20 dogs in this series. The pancreas of these animals when examined both grossly and microscopically was normal.

The effect of an intraperitoneal injection of 2.5 cc. of a 10% solution of bile salts was then studied in a series of 20 dogs, and extensive areas of fat necrosis were observed in all instances. A peritonitis identical with bile peritonitis was produced and fat necrosis was observed in the fat near the pancreas, in the omentum, mesentery and the retroperitoneal fat. Gross and microscopic examination of the pancreas failed to find any evidence of necrosis, distension of the ducts or an inflammatory exudate.

Inasmuch as *B. welchii* was cultured from the peritoneal exudates of the peritonitis produced by both bile and bile salts, the effect of an intraperitoneal injection of cultures of this organism was studied. When 20 cc. of an 18-hour broth culture of *B. welchii* was administered, a peritonitis identical with bile peritonitis was produced; however no fat necrosis was observed.

The question then arose as to whether the bile salts *per se* were able to produce fat necrosis or whether the necrosis was produced by pancreatic enzymes liberated by the local action of the bile salts on the pancreas. In a series of 6 dogs the pancreas was completely

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* This work was done under a grant from the Douglas Smith Foundation of Medical Research of the University of Chicago.

excised before administering intraperitoneally 2.5 cc. of a 10% bile salt solution per kilo. Even though a peritonitis identical with bile peritonitis was produced no fat necrosis was observed.

The data in this investigation tend to show that bile free in the peritoneal cavity will produce fat necrosis. Experiments with bile salt solutions show that the bile salts are the active agents in the bile. Although there is no evidence to show that bile salts *per se* will produce fat necrosis there is evidence to show that bile salts free in the peritoneal cavity and the presence of the pancreas are essential for its production. Inasmuch as there is no gross nor microscopic evidence of pancreatic necrosis, it is necessary to postulate that the pancreatic enzymes were liberated by permeability changes produced by the local action of the bile salts on the pancreas.

5193

Effect of Stasis on the Calcium Content of the Bile.

EDMUND ANDREWS AND LEO HRDINA.

From the Department of Surgery of the University of Chicago.

When bile is stored in the gall bladder its solid constituents undergo a 6 to 8 fold concentration due to the absorption of water. This should produce very high calcium levels theoretically, unless some of the calcium was also absorbed. The solution of this problem is of interest on account of the calcium content of gall stones.

Dogs were anesthetized with ether, the abdomen opened and the cystic duct clamped immediately to prevent emptying of the gall bladder. Then the common duct was opened, cannulae inserted and sufficient liver bile collected for analysis. In 5 experiments the average content of the liver bile on fasting animals was 14.6 mg. per 100 cc. while the simultaneous gall bladder bile contained 52.2 mg. per 100 cc. This is a concentration of about three and a half times, considerably less than the total concentration of the bile, indicating that some calcium as well as water had been absorbed.

The average concentration of normal cystic duct bile in 10 control electrocuted fasting animals was 47.7 mg. In 11 animals in whom the cystic duct had been ligated for varying periods the average calcium content was 39.2 mg. per 100 cc. indicating that a progressive absorption of calcium was taking place. These figures do not truly represent the amount of absorption as the total volume of the gall bladders was always much less after cystic duct ligation. This of course could not be measured quantitatively but it was grossly quite apparent.

Jaundice had no effect on the procedure. When common duct ligation as well as cystic was done the average for the series was 41.6 mg. per 100 cc. Common duct ligation alone leaving the cystic duct open gave an average of 39.9 mg. per 100 cc. for the cystic bile.

Studies on the gall bladder from dogs with cystic duct ligatures were made at intervals of 2 months. In the later observations in each case the calcium content had been reduced 40-50%.

Some of our dogs showed severe inflammatory changes in the gall bladder, and 4 developed actual empyema. In these the bile calcium was lowest of all the series falling within the range of normal liver bile.

The experiments recently reported by Wilkie[1] indicated that in rabbits experimental infectious cholecystitis gave rise to cholesterin stones if the cystic duct was open and to calcium ones if it was ligated. Our experiments were done on dogs as rabbits' gall bladders are too small to get adequate samples for chemical study. They certainly do not indicate that there is a concentration of bile in the gall bladder after cystic duct ligation.

5194

Simultaneous Direct and Indirect Blood Pressure Determinations.*

H. M. LIVINGSTONE AND W. E. ADAMS. (Introduced by E. Andrews.)

From the Department of Surgery, University of Chicago.

In experiments on dogs, Erlanger[1] found that the direct blood pressure in the femoral artery was but slightly higher than the indirect pressure. Kolls[2] and Allen[3] found very little difference between the results of cannulation and the auscultatory method of blood pressure determination on both normal unanesthetized and etherized dogs. Rühl[4] found the direct carotid pressure in rabbits to be fairly comparable to the bleeding time of the vessels of the leg.

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[1] Wilkie, A. L., *Brit. J. Surg.*, 1928, **15**, 450.

* This work has been conducted under a grant from the Douglas Smith Foundation for Medical Research of the University of Chicago.

[1] Erlanger, J., *Am. J. Physiol.*, 1915-16, **39**, 401.

[2] Kolls, A. C., *J. Pharm. and Exp. Therap.*, 1920, **15**, 443.

[3] Allen, F. M., *J. Met. Research*, 1923, **4**, 431.

The following experiments are reported because of the marked variation found between direct and indirect blood pressure determinations.

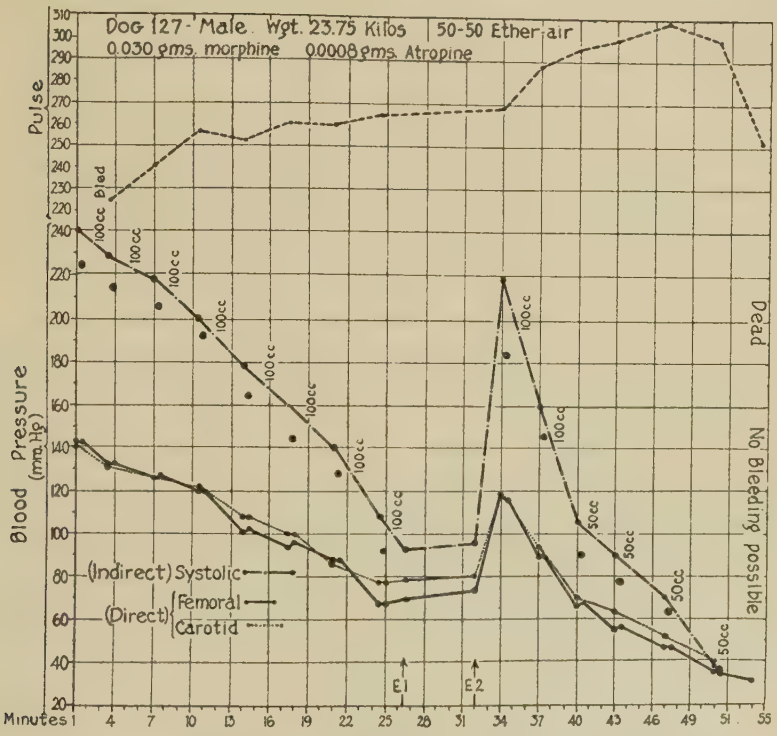

FIG. 1.

Direct and indirect blood pressure and pulse curve during the production of shock.

⊙ Indicates points where bleeding began.

E_1 Intramuscular injection of 150 mgm. ephedrine.

E_2 Intravenous injection of 150 mgm. ephedrine.

Etherized dogs varying in weight from 6.6 to 29.4 kgm. were used. Direct blood pressure readings were obtained from both left carotid and left femoral arteries, which were connected with mercury manometers and a kymograph. A sphygmomanometer cuff and stethoscope bell were used on the left hind leg to determine the auscultatory indirect blood pressure. In 14 experiments the indirect readings were from 20 to 100 mm. of mercury higher than the direct. Observations of the beginning flow of blood from the femoral artery during gradual deflation of the cuff were recorded. Bleeding occurred at a pressure below but much nearer the indirect than the direct. Thus there was a transmission of sound through the artery after the disappearance of bleeding. When shock was produced by intermittent bleeding from the carotid artery, the indirect pressure readings tended to more nearly approximate the direct readings.

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[4] Rühl, A., *Arch. f. Exp. Path. Und Pharmakol.*, 1929, **139–40**, 257.

5195

Bilateral Localization of Prospective Thyroid in the Early Chick Blastoderm, Studied in Chorio-Allantoic Grafts.

DOROTHEA RUDNICK. (Introduced by B. H. Willier.)

From the Hull Zoological Laboratory, University of Chicago.

The vascularized chorio-allantoic membrane of the chick is considered a practically neutral medium in which potencies of embryonic areas may be tested. This technique of chick embryology has been described by Willier.[1]

Histological examination of grafts of the whole pellucid area of blastoderms of the head-process stage, allowed to remain 8 or 9 days on the host membrane, shows normal or almost normal differentiation of a great many tissues: among these thyroid.[2] Embryonic organization at this stage is better revealed in grafts of definite isolated parts of the blastoderm. The study* to be reported has been made on such transplants with regard to the thyroid.

Two types of graft from the head-process stage have been analyzed with reference to differentiation of the thyroid. In the first series, the area pellucida anterior to the level of the primitive node was divided by a median cut into right and left halves: these pieces were grafted separately. In the second series, the same area was divided longitudinally into 3 parts. An attempt was made to keep the width of the median piece that of the node, or slightly greater. In this series, left, median, and right pieces were transplanted separately.

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[1] Willier, B. H., *Am. J. Anat.*, 1924, **33**, 68.

[2] Willier and Rawles, *J. Exp Zool.*, in press.

* The present investigation was aided by a grant to the University of Chicago from the Rockefeller Foundation. I have also the privilege of here acknowledging indebtedness to Dr. B. H. Willier, who originated the problem, and under whose direction its investigation is proceeding. The preparations reported as Series 1 were lent by Dr. Willier.

Series 1. In this series, 5 pairs of grafts, each pair consisting of a right and a left half originating from a single donor, have been examined. Of the 5 left halves, each one contains from one to 3 masses of thyroid tissue. The right halves show less uniformity in this respect. Of the 5, three unquestionably contain thyroid. There is one doubtful case; and one negative. The fact that in 3 indubitable cases out of 5 both grafts of a pair contain thyroid, is conclusive proof that there is prospective thyroid material in both halves of the blastoderm at the head-process stage.

Series 2. The separate transplantation of left, right, and median pieces of the anterior part of the area pellucida of the head-process stage was an experiment designed to give further information as to the localization of the prospective thyroid material at this stage. The results, in part inconclusive, follow:

Of 9 grafts of left pieces examined, 5 contain thyroid tissue (a sixth case is questionable). Of these 5, three may be correlated with successful grafts of median pieces obtained from the same donor. None of these median grafts contains thyroid. This is true of all median grafts examined (ten in all), although they are well-grown grafts containing many other tissues. Data for the right side of the blastoderm are inadequate. Of 5 grafts examined, none contains thyroid tissue. Only one of these grafts is at all well-grown. This lack in the material must be remedied before any conclusions can be drawn in regard to differences in potentiality between right and left sides. That there is prospective thyroid on the right side is proved without question by the results reported above (Series 1). Since no thyroid develops in grafts of median pieces, prospective material on the right side, as well as on the left, must perforce be laterally placed.

The thyroids differentiating in the above grafts show variation in size and shape as well as in histological organization . They appear as more or less compact cell masses of specific character, easily distinguishable from surrounding tissue. As has been indicated, more than one such mass may be present in a graft. The mass may be simple or lobed; flat or rounded; its length may be from 100 to 900 micra. Almost invariably, it is continuous with pharynx or oral cavity wall. It tends to be found in the vicinity of heart (with one possible exception, a constant component of grafts containing thyroid) and of liver. Ganglion cells may be associated with it. The usual appearance of such a thyroid in section is cord-like, with mesenchyme and blood-cells between the cords. Irregular or regular follicles, with walls one cell thick, are frequently seen; occasionally eosinophile colloid is present in the follicles.

The results described briefly above indicate rather clearly that the chick blastoderm contains anterior to the node at the head-process stage, 2 distinct laterally placed areas of prospective thyroid material. In view of the fact that the thyroid arises morphologically as an unpaired median organ, this dual origin is of exceeding interest. The question of whether the thyroid is segregated or determined at this stage; and of whether the right thyroid area differs from the left; cannot at present be answered.

5196

Effect of Histamine Upon the Salivary Flow Induced by Pilocarpine in Dogs.

NATHANIEL KLEITMAN.

From the Department of Physiology, University of Chicago.

In the course of an investigation of the variations in the flow and composition of saliva secreted in response to different types of stimuli, histamine was tried among other pharmacological agents, chiefly because of reports in the literature on its effects upon the salivary glands. These reports refer to acute experiments upon anesthetized or decerebrated animals. Our experiments were made on unanesthetized dogs with permanent fistulae of one of the submaxillary glands, and, aside from obvious advantage of using such animals, it made it possible to repeat an experiment on the same dog several times under identical or different conditions. The 3 dogs used had been accustomed to being placed in a stand and having a measuring tube attached to the lower jaw.

Subcutaneous injections of 1 to 5 gm. of histamine sulphate were not followed by any secretion of saliva. To bring out the possible effect of histamine upon the salivary flow elicited by other means, the dogs were given subcutaneous injections of 1 mg. histamine sulphate, followed 20 minutes later by a subcutaneous injection of 1/3 mg. pilocarpine sulphate per kg. The quantity of saliva secreted under these conditions was compared with that elicited by a similar dose of pilocarpine, not preceded by histamine. This was repeated several times on each dog, histamine-pilocarpine alternating with pilocarpine only experiments. The results in all 3 dogs indicate that a previous injection of histamine inhibits the flow of saliva which follows the administration of pilocarpine. Thus in one dog pilocarpine alone caused a flow of saliva from the fistula amounting on different occasions to 54.9, 48.9, 39.7, and 46.9 cc. per hour (average, 47.6 cc.), whereas in experiments with pilocarpine after histamine, the corresponding amounts of saliva were 31.9, 26.6, 37.6, and 33.8 cc. (average, 32.5 cc.). The largest volume of saliva collected in 5 minutes was 12.8 cc. under pilocarpine alone, and 8.6 cc. with histamine preceding pilocarpine.

5197

Effect of Pituitary Extract (Posterior Lobe) on the Concentrations and Distributions of Chlorides and Bromides in the Blood and Urine.

A. R. MC INTYRE AND H. B. VAN DYKE.

From the Pharmacological Laboratory, The University of Chicago.

The mechanism by which pituitary extract causes antidiuresis in unanesthetized mammals has been considered by some workers as chiefly extra-renal, by others as chiefly renal. The experiments reported here were undertaken in the belief that studies of electrolyte distribution (chlorides and bromides) between erythrocytes and serum might support or oppose the hypothesis that an extra-renal mechanism is involved.

All experiments were performed on unanesthetized dogs with permanent fistulae of only the ureteral portion of the urinary bladder for the accurate collection of urine. Blood was withdrawn from the jugular vein under oil, defibrinated and then centrifuged to separate the serum from the erythrocytes. The serum and the erythrocytes were transferred to separate tonometers without exposure to air. Determinations of the hydrogen-ion concentration of serum and of the water-content and concentrations of chlorides or of chlorides and bromides in urine, erythrocytes and serum, were made 3 or 4 times (including a control-period) in each experiment. Posterior-lobe pituitary extract was given subcutaneously (except in control experiments) in an amount sufficient to cause a typical antidiuretic effect.

The results obtained under the experimental conditions described above appear to justify the following conclusions: 1. Pituitary extract does not significantly change the concentrations of chloride in erythrocytes or in serum of intact (13 experiments on 5 dogs) or of nephrectomized dogs (2 dogs). 2. When part of the blood-chloride has been replaced by bromide, pituitary extract causes no change in the concentrations of chloride and of bromide or in the distributions of chloride or of bromide between erythrocytes and serum of intact (14 experiments on 6 dogs) or of nephrectomized dogs (5 dogs). The *relative* concentrations of bromides and chlorides are about the same in serum and urine (14 experiments on 6 dogs). 3. Pituitary extract causes no change in the water-content of erythrocytes or of serum (34 experiments).

5198

Causation of B. Welchii Infection in Dogs with Sterile Liver Extract and Bile Salts.

EDMUND ANDREWS, ALLAN G. REWBRIDGE AND LEO HRDINA.

From the Department of Surgery of the University of Chicago.

In a recent paper Rewbridge[1] reported that the intraperitoneal injection of sterile bile or pure bile salts brought about prompt invasion of the peritoneum by *B. welchii.* Andrews[2] also showed that autolysis of sterile liver in the peritoneum also provoked a severe *B. welchii* infection. In view of the above and of the work of Dragstedt[3] on the rich flora of the normal liver as well as that of Arnold[4] on the absorption of bacteria into the lymph stream under physiological conditions, our studies were continued and may be summed up as follows. Thirty-nine dogs were used in the series of experiments, 3 dogs in each of the groups.

Experiments were done in which the bile of dogs was led in tubes into the depths of the pelvis and also into the chest cavity. The results were similar. Severe *B. welchii* infections took place within 24 hours in most cases. These experiments showed that the proximity of the intestinal tract or the liver (known foci with rich flora) was not necessary to provoke these infections.

Next, concentrated solutions of liver extract prepared as mentioned in a previous paper[2] and 10% solutions of bile salts were injected into normal dogs into the pelvic peritoneum and also into the chest. In each case these sterile injections *caused violent* anaerobic infections and death in the course of 12 to 20 hours, indicating as in the above experiments that the infection probably took place locally and not by invasion of bacteria from distant sources.

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[1] Rewbridge, Allan G., and Hrdina, L. S., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 528.

[2] Andrews, E., and Hrdina, L. S., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 987.

[3] Ellis, J. C., and Dragstedt, L. R., *Arch. Surg.*, 1930, **20**, 8.

[4] Arnold, Lloyd, *J. Hyg.*, 1929, **29**, 82.

In view of the findings of Reith[5] of anaerobes in normal muscle the following experiments were done. Intramuscular injections of both sterile 10% bile salts and of liver extracts were made under the scapulae in dogs. In most cases within 18 hours there was present an obvious gas gangrene of the adjacent muscular tissue. Emphysema could be felt before the skin was opened. Autopsy showed numerous small gas bubbles, the characteristic odor and greyish gangrenous muscle and culture showed many *B. welchii.* Next specimens of muscles excised under sterile precautions were ground and implanted into dogs' abdomens. Marked septic peritonitis ensued with death in less than 24 hours. Autoclaved ground muscle in like amounts produced no symptoms whatsoever, showing that in this case the infection was probably present in the normal muscle. To settle this point experiments were done in which dogs received barbital in sufficient doses to keep them asleep for 24 hours. One leg was ligated with hay wire so tightly that the arterial circulation was entirely cut off. In the control group simple gangrene of the leg took place, without gas formation, odor or positive culture in most cases. In another group sterile liver extract was injected into the tied off leg, and in each of these cases there was typical gas gangrene, emphysema, typical odor and positive cultures of *B. welchii.*

In view of the fact that in the above experiments the legs are known to contain *B. welchii* and do not develop gas gangrene unless the provocative injection is made, one is led to assume that the mechanism is to be explained on the basis that bile, bile salts, liver extract and perhaps many other substances cause a lowering of the bactericidal power of tissues to such an extent that the bacteria which are always present, either dying or encapsulated in cells, are allowed to grow and to cause actual clinical infection.

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[5] Reith, A. F., *J. Bact.*, 1926, **12**, 367.

5199

Treatment of Peptic Ulcer with Gastric Mucin.

SAMUEL J. FOGELSON. (Introduced by A. C. Ivy.)

From the Department of Experimental Surgery, Northwestern University Medical School.

In the literature on gastric secretion, the capacity of mucus to lower the free acid in the stomach is repeatedly suggested. While working on the "Relationship between Gastric Secretion and Basal Secretion of the Stomach" Lim determined that the concentration of the mucus in the stomach is highest at the lower secretion rates. The problem here was to determine the effect of increasing the mucus content of the stomach upon the free HCl. To evaluate the antacid effect of gastric mucin, it was necessary to either stimulate the secretion of mucin or to administer mucin by mouth. In these experiments attempts at stimulation failed to give consistent results, therefore a neutral preparation of hog mucin was prepared. Two ounces of this mucin in the stomach of Pavlov pouch dogs, after stimulation with 1 mgm. of histamine, was found sufficient to keep the stomach free of free HCl despite the fact that the pouches in these dogs showed adequate gastric secretion. Free HCl would always be present if the same experiment were performed and egg albumen, gelatin or meat substituted for the mucin. When half an ounce of mucin mixed with a pound of meat was fed the dogs, no free HCl was present during an observation period of 5 to 7 hours.

Since January, 1930, twelve patients with definite ulcer histories and typical roentgen findings of peptic ulcer were available for study. In addition to the customary bland diet, one ounce of powdered mucin was added to each meal and about 1 gm. of mucin in tablet form given hourly. All these patients were relieved of subjective symptoms within 3 days of treatment and as yet there has been no recurrence of the pain in observation periods of 2 to 5 months.

Pacific Coast Section.

University of California Medical School, October 15, 1930.

5200

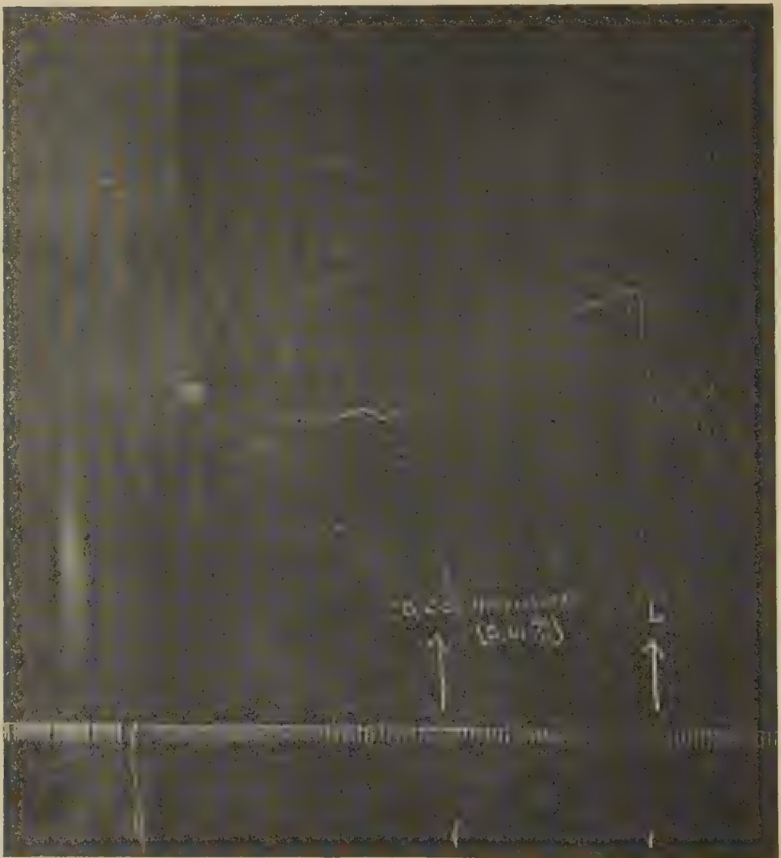
Effect of Vitamin C Deficiency on Uterine Smooth Muscle in Sensitized and Non-Sensitized Guinea Pigs.

SAMUEL H. HURWITZ AND ELIZABETH G. NICHOLLS.

From the Departments of Medicine and Pharmacology, Stanford University School of Medicine.

During a study of various factors on the mechanism of anaphylaxis in the guinea pig, we observed that excised strips of the sensitized guinea pig uterus suspended in warmed Locke's solution failed to respond upon the addition of antigen. That this loss in tonus is not limited to antigenic substances was shown by the failure of the same uterine segments to respond to pharmacologic agents such as histamine, pituitary extract and barium chloride which are powerful stimulants of normal uterine muscle. The effect of a vitamin C deficient diet on the smooth muscle of the bronchi and its relation to the mechanism of anaphylactic shock will be presented in a later study. This paper records the evidence for the conclusion that the removal of vitamin C from the diet produces a loss of uterine smooth muscle reactivity.

Virgin female guinea pigs weighing 250 to 350 gm. were used. Animals of this weight are particularly suited to these experiments because the responses of the sensitized uterine segments in them are more constant and also because experimental scurvy can be produced in such animals in a shorter period of time. The diet used in our observations was not fundamentally basic, but calculated to be fairly complete from a nutritive standpoint except for vitamin C. It consisted of rolled oats and pasteurized milk, a little bran being added to prevent possible intestinal obstruction from the hard fecal masses produced by the oats and milk. In some instances evaporated milk was substituted for pasteurized milk with no change in the results. The amount of milk was not limited. The animals did well on this diet until the beginning of the third week when they began to show some loss of appetite and a considerable loss of weight, amounting in some of the experiments to over 50% of the initial body weight. In spite of this inanition the animals continued moderately active, suggesting that little functional change had been produced in the skeletal musculature. The pathological lesions resulting from such a diet were analogous to those described by previous workers, notably Cohen and Mendel,[1] McCollum and



Control observation showing response of uterine muscle of an animal on a normal diet to a 1-2,500,000 dilution of histamine.

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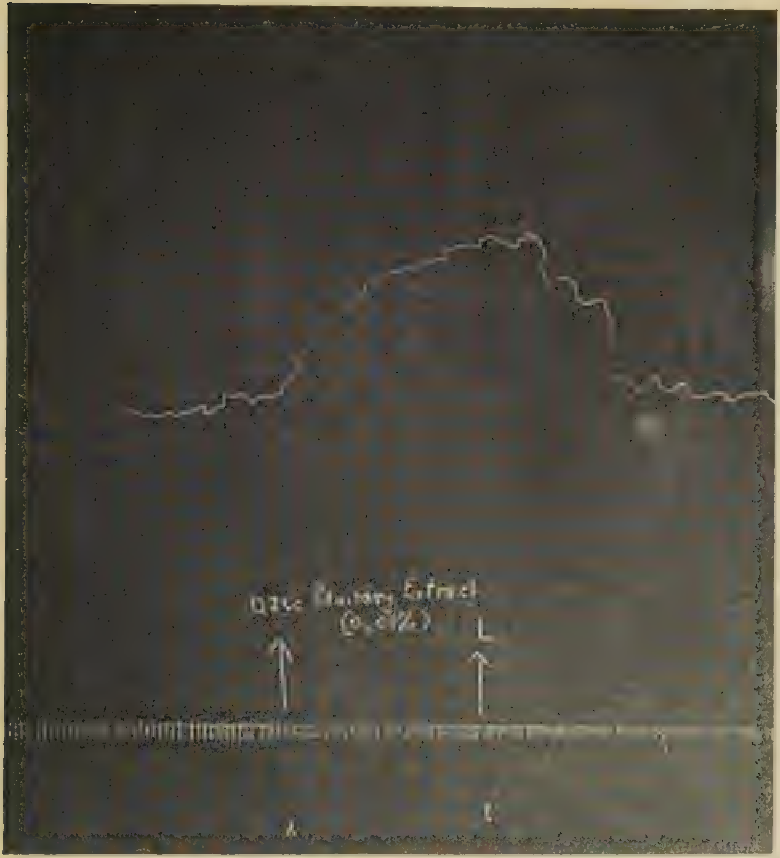
[1] Cohen, Barnett, and Mendel, LaFayette B., *J. Biol. Chem.*, 1918, **35**, 425.



Control observation showing response of uterine muscle of an animal on a normal diet to a 1-2,500,000 dilution of pituitary extract.

co-workers,[2] and Meyer and McCormick.[3] Hemorrhagic effusions were the most characteristic and constant finding. These were found in the skin, the subcutaneous tissues, under the periosteum of the long bones, and in the intestinal tract. Bleeding from the gums was only rarely observed. The most constant lesions were subcutaneous and muscular hemorrhages about the joints of the hind legs and a tendency to epiphyseal separation of the bones, usually present even though the animals showed no subcutaneous hemorrhages.

The uterine horns of these animals were strikingly pale and flabby

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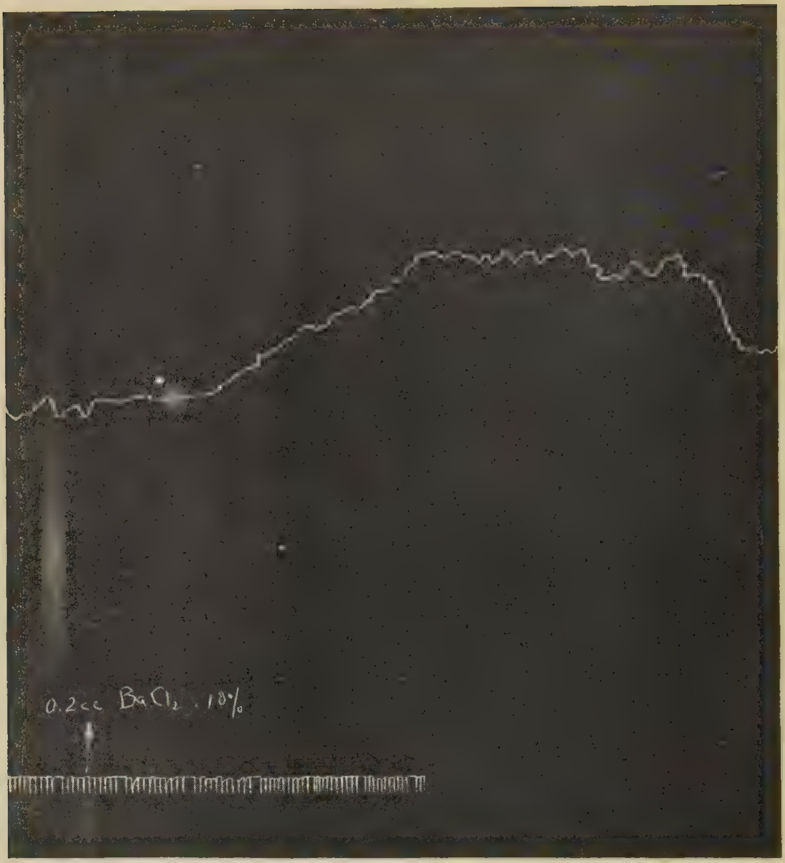
[2] McCollum, E. V., and Simmonds, Nina, "The Newer Knowledge of Nutrition," Macmillan Company, 1929.

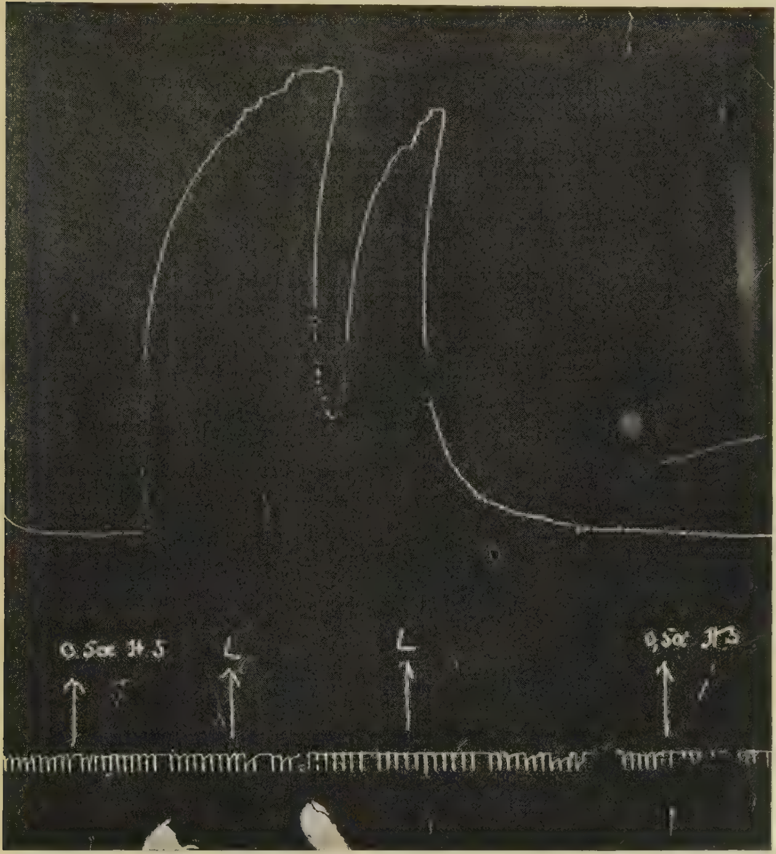
[3] Meyer, A. W., and McCormick, Lewis M., "Studies in Scurvy," Stanford University Press, Medical Sciences, 1928.



Control observation showing response of uterine muscle of an animal on a normal diet to a 1-3,000 dilution of barium chloride.

and stringy. Sections of several such horns showed an uneven staining and an indistinct appearance of the muscle. Many small degenerative areas were noticed. In some sections the nuclei were particularly affected in this degenerative process and in others the muscle fibers. There was considerable vacuolization due probably to fatty degeneration.

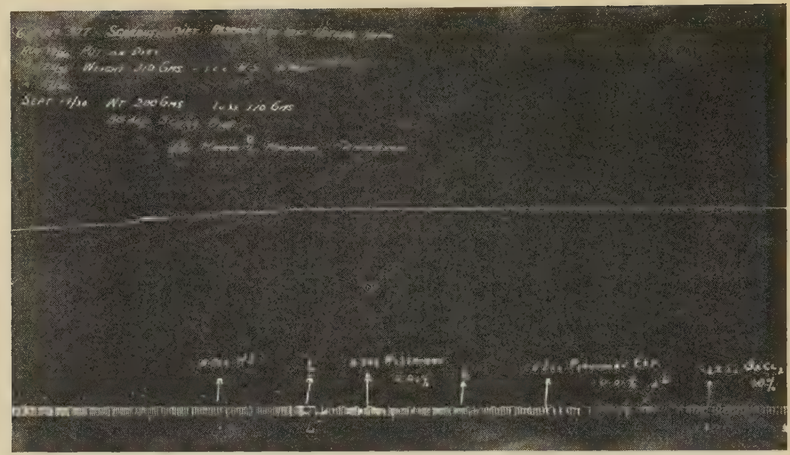
Observations were made on the responses of the uterine segments of 7 sensitized and 9 non-sensitized animals. Only 2 of the sensitized uterine segments gave slight responses on the addition of horse serum. In these instances the vitamin C deficient dietary had produced only a very slight loss of weight. These animals were of a group weighing between 300 and 400 gm. Where heavier guinea pigs are used the scorbutic lesions are produced more slowly and



Guinea Pig 376. Response of left uterine horn of a control animal on a normal diet to horse serum 33 days after sensitization with 1 cc. of horse serum intraperitoneally. The graph shows the desensitization of the muscle following a second injection of serum. The letter L represents the point at which the muscle was washed in Locke's solution.

this may account for the slight responses obtained in them. In the non-sensitized group graphic records were made of the uterine responses of 13 segments to high dilutions of histamine, pituitary extract and barium chloride. Whereas control observations showed typical and very marked responses to 1-2,500,000 and lower dilutions of histamine and pituitary extract and to 1-3,000 and lower dilutions of barium chloride, the uterine smooth muscle of pigs on a diet deficient in vitamin C failed to show any response to dilutions as low as 1-50,000 of histamine and pituitary extract and 1-1,000 of barium chloride.

The significance of these observations in their relation to anaphylactic shock will be recorded in a subsequent study. Inasmuch as the



Guinea Pig 377. Response of left uterine horn to horse serum, histamine, pituitary extract and barium chloride after 30 days on a scorbutic diet, and 25 days after sensitization. Histamine and pituitary extract were used in dilution of 1-2,500,000 and barium chloride in dilution of 1-3,000.

functional pathology of body tissues removed from animals on a diet deficient in vitamins has been little if at all studied, we felt that the recorded observations on the loss of smooth muscle reactivity in such animals are of interest. We have, as yet, no proof that other vitamin deficiencies may not produce similar results. Experimental work is in progress to determine what effect chronic anaemia and inanition in themselves might have on smooth muscle responses to antigens and to pharmacological agents such as those used in this study. Such observations are important because these two conditions are striking clinical accompaniments of vitamin C deficiency.

This loss of smooth muscle reactivity in animals on a diet deficient in vitamin C may result from degenerative changes in the muscle cell or from a loss of intercellular cementing substance[4] or from both lesions.

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[4] Aschoff, L., and Koch, W., *Scorbut—Eine Pathologish—Anatomische Studie*, Gustav Fischer, Jena, 1919.

5201

Rate of Urinary Arsenic Excretion After Giving Acetarsone ("Stovarsol") and "Carbarsone" by Mouth.*

MEI-YÜ CHEN, H. H. ANDERSON AND C. D. LEAKE.

From the Pharmacological Laboratory of the University of California Medical School.

As part of the necessary pharmacological evaluation of a new drug[1] before it should be tried even in controlled clinical experimentation, its rate of absorption (if given by mouth) and excretion should be studied. If possible this should be done on normal human volunteers in order that any deleterious effects may be noted to be guarded against when used in patients. Especially is this procedure important in order to judge the danger of cumulative effect before venturing to employ arsenical compounds on repeated administration in human therapy.

Except for the bare statement that 3-acetylamino-4-hydroxyphenylarsonic acid (acetarsone N.N.R. or "stovarsol") is excreted in the urine after oral administration,[2] we have not been able to find any information regarding its absorption or excretion, in spite of the fact that it has considerable toxicity[3] and has been widely exploited in human therapy with some incidence of poisoning.† The present study was undertaken to supply this deficiency in part. Since our laboratory investigation of a considerable number of arsenical compounds revealed several which would seem on experimental evidence to be superior to acetarsone in treating amebiasis, we decided to include in this study the one upon which we had the most satisfactory data. This was 4-carbaminophenylarsonic acid,‡ previously shown on comparison with acetarsone to have a lower toxicity on oral administration, but more amebacidal action *in vitro,* and with a calculated therapeutic index about 8 times as great.[4]

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* This note is part of an extended cooperative study of the chemotherapy of amebiasis conducted by the Pacific Institute of Tropical Medicine of the Hooper Foundation for Medical Research and the Pharmacological Laboratory of the University of California Medical School, San Francisco, and supported in part by Eli Lilly and Co., Indianapolis, and the Ciba Co., Inc., New York City.

[1] Leake, C. D., *J. Am. Med. Assn.*, 1929, **90**, 1632.

[2] Findlay, G. M., "Recent Advances in Chemotherapy," Phila., 1930, p. 92.

[3] Anderson, H. H., and Leake, C. D., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 267.

† It is our opinion that drug houses should be held responsible for the untoward effects of new drugs in human therapy, if commercial exploitation is made without the previous publication from disinterested sources in reputable scientific journals of data obtained in the pharmacological evaluation of such drugs, especially in regard to toxicity and rate of excretion.

Excretion in the urine is evidence of absorption from the gut following oral administration. The rate of excretion (assuming all the arsenic to be excreted that is absorbed) then would be, for these compounds, an index of the rate of alimentary absorption, and might give some indication of the degree of systemic action to be expected. Direct determination by stool examination of the amount of arsenic not absorbed from the gut is too difficult to be practical. But we intend to control our data on the rate of excretion in the urine, as an index of the rate of absorption of these compounds from the gut, by comparison with the rate of excretion following the intravenous injection of known amounts, and by determining in animals the ratio between the average lethal dose of each compound when given by mouth or intravenously.

The method of analysis was essentially the same as that followed by Young and Muehlberger in studying the excretion of tryparsamide.[5] Following Kjeldahl digestion of aliquot urine samples with sulphuric and nitric acids and neutralizing with sodium hydroxide and sodium carbonate, the arsenic present was titrated by iodine using starch as an indicator.

In one subject undergoing a course of treatment with acetarsone for amebiasis, a total of 11 tablets, each containing 0.25 gm. of the drug, were given over a period of 6 days. Of a total of 0.756 gm. of arsenic administered, 29% was recovered from the urine in 14 days following the beginning of treatment. In a second subject receiving 14 tablets of acetarsone (for amebiasis) over a period of 7 days, 17% of the 0.952 gm. of arsenic administered was recovered during the 10 days after treatment began. In a normal human (A) receiving two 0.25 gm. tablets of acetarsone containing 0.138 gm. of arsenic, 20% was recovered in 72 hours after giving the drug, but in a second subject (L) given the same amount, only 7% was found in 29 hours following oral administration. Two months later, these same 2 normal subjects were each given 0.5 gm. "carbarsone" by mouth, containing 0.135 gm. of arsenic. From subject L, 13% of the arsenic was recovered from the urine after 42 hours, while only 8% was found in the urine of the other subject (A) after 52 hours. The rate of excretion of the 2 drugs in the normal subjects is shown graphically in Fig. 1.

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‡ Deutsches Reichpatent, 213155; U. S. P. No. 937929 (issued to Paul Ehrlich and A. Bertheim, 1909); supplied by the Lilly Research Laboratories. For convenience in expression we propose to call this substance "carbarsone."

[4] Leake, C. D., Koch, D. A., and Anderson, H. H., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 717.

[5] Young, A. G., and Muehlberger, C. W., *J. Pharmacol. Exp. Therap.*, 1924, **23**, 461.

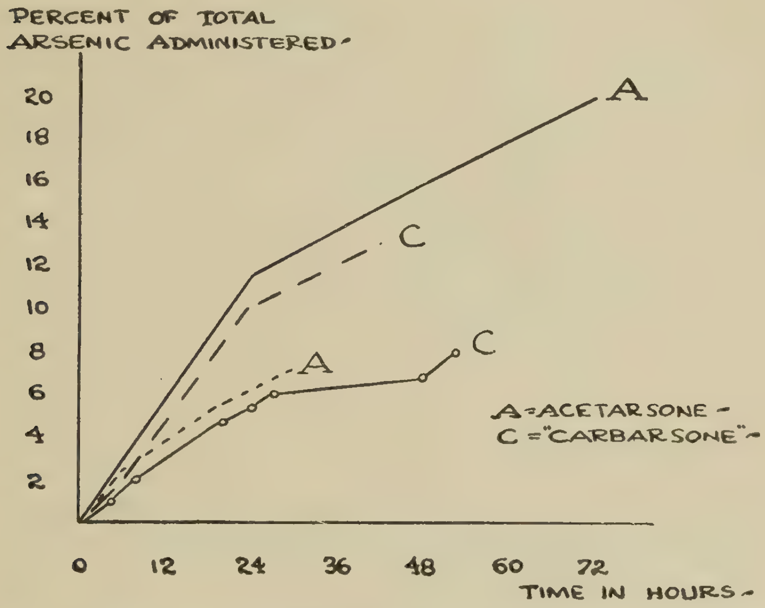

FIG. 1.
Rate of urinary excretion of arsenic after oral administration of acetarsone and "carbarsone" in normal humans.

The fact that in the normal subjects from 10 to 30% of the recovered arsenic appeared in the urine within 6 hours after ingestion is evidence of fairly prompt absorption. Although 60 to 75% of the total arsenic found in the urine was eliminated within 24 hours after ingestion, the delay in the excretion of the remainder recovered may be explained on the basis of delayed absorption as the drug passes down the gut. Nevertheless this delay presents an element of danger in connection with the possibility of cumulative systemic effect on repeated administration. Especially is this liable with "carbarsone", where the substituted amino group in the para position to the arsenic suggests possible deleterious action on the optic tract.[6] Even though this drug would probably not be used in human therapy (for amebiasis) in oral doses larger than 0.5 gm. per day for 10 days

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[6] Young, A. G., and Loevenhart, A. S., *J. Pharmacol. Exp. Therap.*, 1924, **23**, 107.

(the total of which would equal a frequently used single intravenous dose of tryparsamide, a closely related compound), careful watch for eye symptoms would be a necessary phase of the treatment. No symptoms of any sort were noted in our normal subjects.

Summary. 3-acetylamino-4-hydroxyphenylarsonic acid (acetarsone N.N.R. or "stovarsol") and 4-carbaminophenylarsonic acid ("carbarsone") are rather slowly excreted in the urine after oral administration. In one normal human subject 20% of the ingested arsenic in 0.5 gm. acetarsone was recovered in the urine in 72 hours, but only 8% of the arsenic of the same dose of "carbarsone" after 52 hours. In another normal human, 7% of the arsenic received in the same dose of acetarsone was found in the urine in 24 hours, while 13% was recovered from the same amount of "carbarsone" in 42 hours. Even though relatively small absorption from the bowel is indicated by these experiments, "carbarsone" should be used cautiously in human therapy until it is established whether or not it is liable to injure the optic tract.

5202

Iodoxybenzoate as a Test Reagent for Free Phenolic Hydroxyl Groups in Organic Compounds.

CHAUNCEY D. LEAKE.

From the Pharmacological Laboratory of the University of California Medical School.

While attempting to study the pharmacological action of oxidation products of morphine, I tried to induce rapid oxidation of the drug by means of ammonium iodoxybenzoate. The salts of iodoxybenzoic acid have been studied by Loevenhart and his associates[1] and their oxidizing properties are well known. Upon treating morphine salts with an aqueous solution of ammonium iodoxybenzoate the straw to garnet color characteristic of a morphine solution on eremacausis develops within a few moments. No other opium alkaloid, except apomorphine, seems to yield color with this reagent. Codeine is monomethyl morphine, with the methyl group replacing the hydrogen of the phenolic hydroxyl group of morphine. This

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[1] Loevenhart, A. S., and Grove, W. E., *J. Pharmacol. Exp. Therap.*, 1911, **3**, 101; Arkin, A., *Ibid.*, 1911, **3**, 145; Young, A. G., and Yeomans, J. B., *J. Am. Med. Assn.*, 1926, **87**, 746.

"muzzling" of the phenolic hydroxyl group (which may explain the difference in action between the drugs), and the resulting inhibition of the oxidation color development on treatment with ammonium iodoxybenzoate, suggested that the reagent might be used for the detection of "free" phenolic hydroxyl groups in various other aromatic organic compounds.

This was found to be the case. The reactivity of the phenols in color development, especially in contact with aldehydes and inorganic acids, has been recently reviewed by Levine and Magiera.[2] The reaction system, phenol-aldehyde-acid, is so powerful, however, that "muzzled" phenolic hydroxyl groups, such as found in codeine or acetyl-salicylic acid, are opened up so that color develops. Hence such tests as Pettenkofer's[3] and Marquis'[4] for the opium alkaloids, for instance, do not differentiate morphine from its associated alkaloids most of which seem to contain "muzzled" phenolic hydroxyl groups. The advantage, then, of iodoxy benzoate as a test reagent in connection with phenolic compounds lies in the fact that it does not decompose the compounds with which it comes in contact, but merely reveals, by an oxidizing color reaction, the presence of a "free" phenolic hydroxyl group. Thus, like Lautenschlager's diazonium reagent,[5] it will not react with any opium alkaloid except morphine and apomorphine. Again, in comparison with an unneutralized arsphenamine solution, it reacts only with extreme slowness with a completely neutralized one, although a precipitate forms at once in both.

Obviously, this oxidation reaction between iodoxybenzoate and "free" phenolic hydroxyl groups in aromatic organic compounds may be made quantitative, and applied to the estimation of the concentration of a solution of unknown strength of a reacting substance by colorimetric comparison with a standard. The test, by virtue of its simplicity, should be useful for such purposes as differentiating morphine and apomorphine from each other and from other opium alkaloids, epinephrine from ephedrine, and acetarsone from other pentavalent arsenicals. It might also be useful for detecting the presence of phenol or cresol as preservatives in organic fluids. For these reaction purposes, a 1% freshly prepared aqueous solution of ammonium iodoxybenzoate allowed to act directly on the material, or in known concentration in solution, is satisfactory.

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[2] Levine, V. E., and Magiera, E. A., *J. Lab. Clin. Med.*, 1927, **12**, 743.

[3] Pettenkofer, M., Medical Jurisprudence, Ed. 2, New York, 1911.

[4] Marquis, E., *Arb. Pharmakol. Inst. Dorpat*, 1896, **15**, 117.

[5] Lautenschlager, L., *Arch. d. Pharm.*, 1919, **257**, 13.

It does not matter whether or not the substance or solvent is miscible with the water solution of the iodoxybenzoate as long as thorough shaking is employed. Carvacrol and eugenol, for example, give powerful color reactions under these conditions, as does thymol in an organic solvent.

Of general biological significance in connection with this "free" phenolic hydroxyl oxidation reaction, is the fact that the pharmacological effect of compounds so reacting does not seem to be altered quantitatively or qualitatively. Thus, as far as I could determine in rabbits and dogs, the effects of freshly made colorless solutions of morphine sulphate and of morphine sulphate solutions in equal concentration oxidized to a deep garnet color by ammonium iodoxybenzoate were the same on subcutaneous injection of equivalent amounts. On intravenous injection the acute reactions peculiar to ammonium iodoxybenzoate occur, of course, with the solutions in which this agent is present. This interfered somewhat with the comparison between unoxidized and oxidized epinephrine in the manner indicated but did not seem greatly to inhibit the blood pressure raising effect characteristic of the drug. In the case of the arsphenamines, Voegtlin and Smith[6] suggest that oxidation of these arsenicals is a necessary preliminary step to the manifestation of their typical biological activity. On the other hand, the "muzzling" of the "free" phenolic hydroxyl group greatly reduces the toxicity and general pharmacological action of the parent substance. Thus codeine, phenetidin, and completely neutralized arsphenamine are less toxic and less "active" than morphine, p-amidophenol, and unneutralized arsphenamine respectively. Two reasons, then, indicate that the characteristic pharmacological actions of aromatic organic compounds containing "free" phenolic hydroxyl groups are mediated by oxidative processes in the body involving these groups; (*a*) oxidation outside the body previous to administration does not interfere with their typical effects; (*b*) "muzzling" of these groups and thus inhibiting their oxidative reactivity does alter their characteristic action, as appears for example in the differences in pharmacological activity between morphine and codeine, or between salicylic acid and acetyl salicylic acid.

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[6] Voegtlin, C., and Smith, H. W., *J. Pharmacol. Exp. Therap.*, 1920, **15**, 475.

5203

The Anesthetic Properties of Certain Unsaturated Ethers.

CHAUNCEY D. LEAKE AND MEI-YÜ CHEN.

From the Pharmacological Laboratory of the University of California Medical School.

From a general consideration of the chemo-pharmacological properties of di-ethyl ether and ethylene, especially in regard to their marked anesthetic power and relatively low toxicity, it seemed possible to predict that compounds combining the chemical characteristics of each would be interesting general anesthetic agents. This prediction might be made more specific by further reference to the theory of the relationship between chemical constitution and pharmacological action. In certain homologous series of absorbable aliphatic compounds (as the monohydric alcohols) toxicity increases (without a comparable increase in *desired* activity), in proportion to the number of carbon atoms in the straight carbon chain. Also related to chemical structure in such a series are certain anesthetically important physical properties, such as boiling points. So, one might venture to anticipate that the higher members of our proposed homologous series of unsaturated ethers would not be found as satisfactory inhalation anesthetic substances as the lower members of the series, because of too high boiling points and too great toxicity.

Through the kindness of Professor Sigmund Fraenkel of Vienna, we obtained vinyl-ethyl ether, allyl-ethyl ether, and di-allyl ether, and through the courtesy of Professor Lauder Jones of Princeton, we secured from Doctor Randolph Major di-vinyl ether, vinyl-ethyl ether, and isopropenyl-ethyl ether. These agents are all volatile colorless liquids with odors varying from the rather pleasant aromatic vapor of di-vinyl ether to the pungent irritation of the allyl compounds. Unfortunately only small quantities of these substances could be supplied (none in excess of 12 cc.), so that only tentative determinations could be made of their partition coefficients and of their action on inhalation in mice.

The partition coefficients (between olive oil and water at 20°C.) of these unsaturated ethers in equimolecular concentrations was estimated, in comparison with ordinary ether, by gravimetric methods, after 15 minutes shaking in small separatory funnels, and after several days standing. The figures obtained by this method were approximated, in the case of substances with relatively low boiling points, by a volumetric technique employing the Van Slyke volu-

TABLE I.
Physico-chemical properties of some unsaturated ethers in comparison with di-ethyl ether.

Substance	Formula	Molecular Weight	Boiling Point	Partition Coefficient, Oil : Water, at 20°C.
Di-ethyl ether	$\left.\begin{matrix}CH_3{-}CH_2\\CH_3{-}CH_2\end{matrix}\right\rangle O$	74	34.5°C.	2.3±0.1
Vinyl-ethyl ether	$\left.\begin{matrix}CH_2{=}CH\\CH_3{-}CH_2\end{matrix}\right\rangle O$	72	34-36°C.	0.5±0.1
Di-vinyl ether	$\left.\begin{matrix}CH_2{=}CH\\CH_2{=}CH\end{matrix}\right\rangle O$	70	36-39°C.	2.5±0.2
Allyl-ethyl ether	$\left.\begin{matrix}CH_2{=}CH{-}CH_2\\CH_3{-}CH_2\end{matrix}\right\rangle O$	86	68-74°C.	2.0 ?
Isopropenyl-ethyl ether	$\begin{matrix}&/CH_3\\CH_2{=}\ C&\diagdown_{O}\\CH_3{-}CH_2&/\end{matrix}$	86	59-63°C.	0.61±0.1
Di-allyl ether	$\left.\begin{matrix}CH_2{=}CH{-}CH_2\\CH_2{=}CH{-}CH_2\end{matrix}\right\rangle O$	98	92-98°C.	2.0 ?

metric gas apparatus. Our tentative and averaged estimations appear in Table I. The figures given for allyl-ethyl ether and di-allyl ether are not satisfactory because only a single determination was possible. With vinyl-ethyl ether, di-vinyl ether, and isopropenyl-ethyl ether, 3 gravimetric estimations were made and averaged, while 10 gravimetric determinations were made and averaged for di-ethyl ether. Di-vinyl ether alone of the unsaturated ethers studied seems to have a slightly higher partition coefficient than di-ethyl ether. Isopropenyl-ethyl ether, the forked chain isomer of allyl-ethyl ether, seems to have a much lower partition coefficient. We were surprised to find such a low partition coefficient for vinyl-ethyl ether. But if anesthetic power is proportional to partition coefficient, di-vinyl ether might be predicted to be the best anesthetic agent of the series.

In attempting to evaluate the anesthetic action of these compounds, we used mice for economy of material. As a standard we used 2 cc. di-ethyl ether allowed to evaporate completely in a 4 liter jar. This yields a molecular concentration of 0.0048 mols per liter of air. The other agents were measured in such amounts as to give equimolecular concentrations on evaporation in their respective containers. With care taken to avoid any asphyxial complications, individual fresh mice were placed in the containers and watched. Observations were made of respiration, general reactions, especially such as might indicate irritation, the length of time required to anesthetize, and the period required for recovery after removal from the chamber.

TABLE II.
Average anesthetic effects on mice of equimolecular concentrations of certain unsaturated ethers in comparison with di-ethyl ether.

Substance	Average time for induction of anesthesia in seconds	Average time for recovery from anesthesia in seconds	Irritation of respiratory mucous membranes	Number of mice used
Di-ethyl ether	98	85	+	15
Vinyl-ethyl ether	440	1140	+++	4
Di-vinyl ether	60	80	0	3
Allyl-ethyl ether	182	104	+++	6†
Isopropenyl-ethyl ether	195	380	++	3
Di-allyl ether	288	160	++++	6*

† One died in convulsions 1 hour after recovery from the anesthesia.
* Four died in convulsions within 4 hours after recovery from the anesthesia.

Our experimental observations on mice are summarized in Table II. Every available anesthetic brand of di-ethyl ether seemed to cause some irritation. Di-vinyl ether, however, appeared free from irritating effect in the concentration used. The other unsaturated ethers were all much more irritating to the mucous membranes of the eyes and respiratory tract than was ordinary ether. Di-vinyl ether not only anesthetized more promptly than did di-ethyl ether in the same concentration, but recovery on removal from the chamber was also more rapid. The other unsaturated ethers were, however, much slower than di-ethyl ether in causing anesthesia and recovery was more prolonged. Vinyl-ethyl ether was surprisingly weak in anesthetic power at the concentration used, although this conformed to its low partition coefficient. The prolonged recovery period required for this compound is difficult to explain. Isopropenyl-ethyl ether was also weak in anesthetic action at the concentration employed, again as suggested by its relatively low partition coefficient. Allyl-ethyl ether, the straight chain isomer of isopropenyl-ethyl ether, while a more powerful anesthetic, began to display the predicted greater toxicity of the higher members of the series. In diallyl ether this toxicity was even more marked, again as was to be expected. Respiration was not greatly altered by the anesthetic concentrations of di-vinyl ether or of di-ethyl ether, but was made very slow and laborious by the other compounds.

In this series of unsaturated ethers a fair degree of correlation seems to exist between their partition coefficients, as we have tentatively estimated them, and their anesthetic power in equimolecular concentration as judged by the time period required to anesthetize. Except for vinyl-ethyl ether, the general relationship between chemical constitution and pharmacological action, as we could predict from present theory, seems to hold. Since the vinyl-ethyl ether used by us was from two independent sources and was found to have the same properties, to account for its marked failure to conform to theoretical behavior is difficult. At any rate, further study of this interesting series of agents is justified and is cordially invited.

Summary. Of a series of unsaturated ethers, including vinyl-ethyl ether, di-vinyl ether, allyl-ethyl ether, and its isomer iso-propenyl-ethyl ether, and di-allyl ether, only di-vinyl ether was found to have a partition coefficient and anesthetic properties on inhalation in equimolecular concentrations in mice approaching or superior to di-ethyl ether. A close correlation was found between the tentative partition coefficients and anesthetic powers of these unsaturated ethers. With the exception of vinyl-ethyl ether, these compounds conformed in general behavior to what was predicted of them on the basis of present theoretical conceptions of the relationship between chemical constitution and pharmacological action.

5204

Effect of Oxidation-Reduction Potential on Some Enzymic Reactions.

SALLY M. FIELD AND JOHN FIELD, II. (Introduced by E. G. Martin.)

From the Department of Physiology, Stanford University.

During the last decade great progress has been made in the study of the intensity factor in oxidation-reduction equilibria. The investigations of Mansfield Clark and others have made possible the quantitative determination of this factor both *in vitro* and *in vivo*.

It has been known for a long time that the activity of the several enzymes essential in life processes is conditioned by a variety of factors, such as temperature, the nature and concentration of electrolytes (notably the hydrogen ion concentration), the dilution of enzyme and of substrate, irradiation.[1, 2, 3, 4, 5, 6, 7, 8, 9] It seems prob-

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[1] Compton, A., *Proc. Roy. Soc.*, 1921, **92B**, 1.

[2] Compton, A., *Ann. de l'inst. Pasteur*, 1916, **30**, 497.

[3] Kawakami, T., *J. Pharm. Soc. Japan*, 1929, **49**, 346.

[4] Kita, G., *Mem. Coll. Eng. Kyoto Imperial Univ.*, 1918, **21**, 5.

able on *a priori* grounds that in the case of some enzymes, the oxidation-reduction potential of the system may be important. The experiments of Stephenson[10] which showed a relation between pH and rate of reduction of lactic acid by dehydrogenase, are interesting in this connection.

We have undertaken the study of enzyme action in buffered systems in which the oxidation-reduction potential is kept constant—precautions not hitherto observed as far as we know. The first case investigated was the hydrolysis of starch by takadiastase.

Known amounts of indicator and of starch were incubated at 37.5° in solutions buffered at pH 5.9 (optimal for takadiastase) and poised at various oxidation-reduction potentials, and the maltose formed in the experimental and control tubes was determined at the end of a 30-minute period. To prevent oxidation of the reduced dyes, the reactions were carried out in an atmosphere of oxygen-free nitrogen, which was first passed through a furnace of finely divided copper heated to 750°C. to remove traces of oxygen. The several gas outlet tubes led to one common tube, to insure equal pressure in all vessels, and the tip of this latter tube dipped in mercury to prevent back diffusion of oxygen. Potentials were followed during the run by the usual method, *viz.,* measurement of the potential difference between a platinum electrode in the solution and a reference calomel half-cell. The reaction tubes were painted on the outside with black enamel to exclude light.

We found that the maltose formed from soluble starch in 30 minutes was the same in systems poised at a low E_h with β-anthraquinone, at intermediate E_h with methylene blue, and at high E_h with quinhydrone.

Two experiments on the system muscle extract plus kidney cortex extract + glucose buffered at pH 7.2 and poised with methylene blue, have shown no difference between the experimental tubes and control tubes which did not contain methylene blue, in respect to glycolysis. The muscle extracts were prepared according to the method of Case and McCullagh.[11] These studies are being continued and extended.

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[5] Nishikawa, K., *Biochem. Z.*, 1927, **188**, 386.

[6] Pincussen, L., and Kambayashi, Y., *Biochem. Z.*, 1928, **203**, 334.

[7] Pincussen, L., and Oya, T., *Biochem. Z.*, 1929, **209**, 410.

[8] Sherman, H. C., and Tauberg, A. P., *J. Am. Chem. Soc.*, 1916, **38**, 1638.

[9] Waldschmidt-Leitz, E., "Enzyme Actions and Properties," 1929.

[10] Stephenson, M., *Biochem. J.*, 1928, **20**, 605.

[11] Case, E. M., and McCullagh, D. R., *Biochem. J.*, 1928, **20**, 1060.

5205

Life History of Protostrongylus (Synthetocaulus) rufescens.

A. and M. HOBMAIER. (Introduced by K. F. Meyer.)

From the Laboratory of the State Fish and Game Commission, George Williams Hooper Foundation for Medical Research, University of California.

Synthetocaulus rufescens produces extensive lung lesions and is economically one of the most important lungworms; consequently a study of the intermediate host was undertaken.

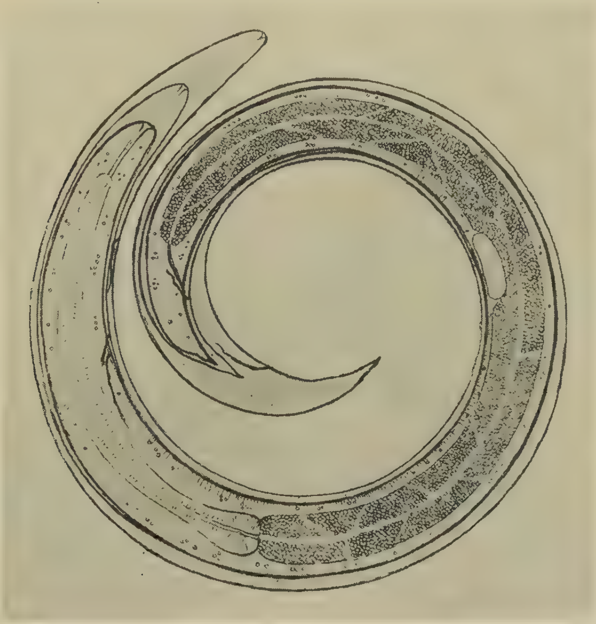
The body of the embryo is small and filariform. The cuticula shows very fine transverse striations. The width of the body is 16-20μ near the centre of the bodily length. Both extremities are gradually tapered. Near the head it is reduced to 8.3μ. The entire embryo measures from 300 to 325μ. The oesophagus measures about 145μ and shows 2 bulbous enlargements corresponding to the embryo of other lung-worms. The opening of the excretory duct is located 87-95μ while the genital rudiment is situated about 200μ from the tip of the head. The intestinal tube breaks through the cuticula 40-42μ from the tip of the tail. The tail is slightly curved and is separated and recognized by a very small dorsal indentation. It is without any appendix on the back.

Biology and Morphology of the Larvae. The viability is the same as that of the embryos of *Mullerius capillaris.* They are rapidly killed by complete desiccation. In clear water they remain alive for more than one year. In artificial culture the presence of infusoria is of little consequence, while rotifera may destroy the embryos in a few days.

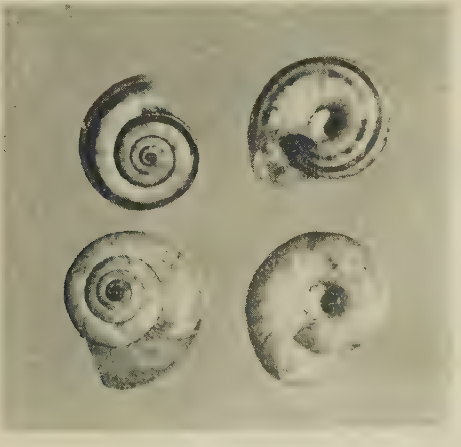
Continuous observations for indefinite periods never reveal changes in the structure of the embryo in the free state or any indications of a transformation into the larval stage. Obviously the embryo has to pass through an intermediate host. In former publications it was shown that the development of the *Synthetocaulus* is completed in several species of snails which act as intermediate hosts. *Protostrongylus (Synthetocaulus) rufescens* probably develops in a similar manner. Several unsuccessful experiments have been conducted with aquatic snails and with geophilous snails. The intermediate hosts of *Synthetocaulus (Mullerius) capillaris* failed to yield larvae of *Synthetocaulus rufescens* when fed with embryos of this lungworm.

However larvae were secured when the experiments were conducted on the genus *Helicella.* It is very interesting that the intermediate hosts of *Mullerius capillaris* are snails which prefer a shaded or half shaded environment, while those of *Protostrongylus rufescens* belong to the dry geophilus snails.

The following species belong to the genus Helicella: *helicella, helicopsis* and *candidula.* Experiments were conducted only with



Protostrongylus rufescens; fully developed larva stage in the dry-geophilus snail *Helicella ericetorum.* Natural size 560-620μ.



Helicella ericetorum, intermediate host of *Protostrongylus rufescens.* Dorsal and ventral face of striated and non-striated form.

Helicella ericetorum, Helicella obvia, and *Hellicella bolli.* Future studies will include other species of Helicella.

The embryos enter the snail by way of the mucous glands of the foot. Histologic sections of the foot reveal the mode of penetration of the embryos and the transformation into larvae. A small tubercle-like proliferation is formed around the worm as an expression of a reaction to the invasion. The development of the embryo into the larva is quite similar to that of *Mullerius capillaris.* It is completed 12-14 days after infection of the snails. Many changes in shape and size of the parasite characterize the 2 moultings. The cuticula appears separated but it is not shed. The twice moulted embryo represents the infective larva.

The middle sheath is usually attached to the larva; but it may also be completely separated or it may form a tail and a head cap when the worm retracts the head or tail. The non-pigmented, transparent larvae show a distinct intestinal tube. The dimensions are as follows: Greatest width of the finely striated body is 40-45μ; the length 560-620μ; opening of the excretory duct 100μ posteriorly to the head; length of the eosophagus 200-220μ, of the intestinal tube 300-350μ, anus to tip of tail 50μ. The genital rudiment approximately 160 to 180μ posteriorly to the eosophageal ending.

The development in the mammals is the same as that formerly described for other lungworms. The moult in the mesenteric glands of the host, and the pathological anatomy will be discussed in a consequent paper.

5206

Poliomyelitis Virus. Results of Treating with Certain Chemicals and with Heat.*

B. HOWITT. (Introduced by K. F. Meyer.)

From the George Williams Hooper Foundation for Medical Research, University of California, San Francisco.

Vinson and Petre[1] demonstrated that the virus causing mosaic disease of tobacco was "in many ways analogous to that of a chemical substance," since it could be precipitated by certain dyes and chemicals without loss of virulence and could be recovered uninjured

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* This study was aided by grants from the anonymous Poliomyelitis Donation of the Hooper Foundation.

[1] Vinson and Petre, *Botan. Gaz.*, 1929, **87**, 14.

after removal of the phosphates, sulphates, pigments and most of the protein contained in the plant juice. It was thought of interest to apply certain of their methods to the virus of poliomyelitis, although the one virus was of plant and the other of animal origin. Certain modifications in technique were necessary, however. Strict asepsis had to be practiced while care had to be taken in neutralizing the end product and in pulverizing any precipitate before inoculation. The necessity of intercranial injection into monkeys required particular attention as to the amount, the character and the toxicity of the substance given.

A Berkefeld V filtrate of a 10% saline suspension of monkey poliomyelitis virus was employed. Definite proportions of the chemicals were added to a standard amount of this filtrate, usually 50 cc. Whenever a precipitate was used, it was repeatedly washed with saline, ground in a sterile mortar with a definite amount of saline and then filtered through sterile gauze before intercranial inoculation. If a supernatant fluid was given the reaction was adjusted to pH 7.0. A control monkey injected with the virus filtrate was always added to each experimental series. Tests for proteins, carbohydrates and occasionally for lipoids were made whenever sufficient material was available. Daily observations, including rectal temperatures were taken on each monkey for 2 to 3 weeks unless the animal developed typical poliomyelitis after the usual incubation of 5-7 days. Histological sections of the cord and brain were examined from all animals succumbing to the disease.

Certain experiments were successfully repeated, while others required numerous attempts before a positive result could be obtained, as indicated by the presence of typical symptoms of poliomyelitis in the monkey: rise in temperature, excitability, tremor, followed by either a partial or complete flaccid paralysis and prostration.

In 2 experiments the poliomyelitis virus was present, both in the supernatant fluid and in the precipitate after treating the filtrate with a solution of 1% aqueous safranin. It was precipitated by the latter after removal of most of the proteins with basic lead acetate.

On 3 occasions the virus was demonstrated in the supernatant fluid after treating the filtrate with basic lead acetate and the further addition of either barium acetate or of sodium bicarbonate. The heat and acetic acid and the ammonium sulphate tests for protein were negative but the ninhydrin was slightly positive in each case.

In 6 experiments the virus was precipitated from the filtrate by equal parts of acetone (Baker's C. P.), while the monkeys injected with the supernatant fluid did not develop poliomyelitis. The ninhydrin tests for protein were either negative or only very slightly positive with these precipitates.

In one experiment the virus was recovered both in the supernatant fluid and in the washed precipitate after treating the filtrate with acetic acid to pH 5.0 and heating to 55°C., while in another the virus was found only in the supernatant fluid after acidifying to pH 4.4 and heating to 58°C. The washed precipitate from the latter was negative. Heating to 71°C., to 75°C., and to 78°C., destroyed the virus.

After a number of unsuccessful attempts it was most interesting to find the virus in the final precipitate after treating the filtrate with lead acetate and sodium bicarbonate and then leaving it on ice over night following the addition of equal parts of acetone. Monkeys injected both with the supernatant fluid after the removal of the major part of the proteins and with the acetone precipitate developed poliomyelitis. The latter gave a negative ninhydrin test.

In the same experimental series a potent virus was obtained in the precipitate which formed following acidification of the filtrate to pH 4.4, heating to 58°C. and holding it on ice over night with equal parts of acetone. The washed acetone precipitate was ninhydrin negative and produced a typical flaccid paralysis in a monkey.

Several attempts were made to extract a potent virus from the acetone precipitate with 95% alcohol and with ether. Both the evaporated extractive material and the residue failed to infect monkeys. Negative results were also obtained after several attempts both to precipitate the virus with different proportions of absolute alcohol and to recover it in the supernatant fluid after adding varying amounts of phosphotungstic acid.

5207

Metabolism Studies on the Brucella Group. II. The Fermentation of Monosaccharides.

C. E. ZOBELL AND K. F. MEYER.

From the George Williams Hooper Foundation for Medical Research, University of California, San Francisco.

It was generally believed that members of the Brucella group lack the power to ferment the common sugars until McAlpine and

Slanetz[1] showed that all the varieties except the bovine *abortus* strains are able partly to decompose and utilize the available glucose in the medium. Meyer and Eddie[2] failed to find any definite correlation between the type of Brucella and glucose utilization. More recently Coleman and coworkers,[3] Mallardo,[4] and Duncan and Whitby[5] found slight acid changes in arabinose, xylose, galactose and levulose. However, the degree of acid production varied with the different strains and could not be correlated with the origin of the cultures.

Since preliminary tests indicated that the chlorphenol red indicator used by Coleman and coworkers is reduced by certain Brucella strains it appeared desirable to test the fermentative properties of a large series of strains by the following procedure: A 1% filtered beef infusion rendered sugar-free by the action of *B. saccharolyte,* 0.5% NaCl, 0.2% agar adjusted to a pH 6.9 was mixed with 1% filtered goat serum and 1% carbohydrate (filtered solutions), tubed in 3.5 cc. amounts, tested for sterility and inoculated with a uniform sized loopful of a slant culture suspended in saline. The tubes were held at 37° C. and the pH of the cultures determined with brom thymol blue according to the method of Clark,[6] on the 3rd, 7th, 10th, 14th, and 21st day of incubation. Changes in the reaction became perceptible in 3 to 7 days, the maximum intensity being reached in 10 to 21 days. Duplicate determinations were made on 194 strains from host origins in 15 countries of the world.

The semi-solid consistency of the medium favored the growth of the bacteria and the low buffer content permitted a rapid change in the H-ion concentration. In the control medium containing no carbohydrate the final reaction was pH 7.6 or above, with all strains. The tests for ammonia were invariably positive. Rhamnose and the alcohol, mannitol, were never fermented as indicated by the pH 7.6. A simultaneous attack of carbohydrate and the nitrogenous compounds was recorded in media containing arabinose, xylose, galactose, glucose and levulose, indicated by a terminal reaction less than pH 7.6 with these sugars. They were attacked with intensity in the order named. The growth energy was not influenced although many strains increased the H-ion concentration to pH 6.0 or below. Regardless of the final pH the production of ammonia could always be demonstrated by making the medium alkaline with NaOH. There is no evidence of a protein sparing action by the carbohydrates.

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[1] McAlpine and Slanetz, *J. Infect. Dis.*, 1928, **42**, 73.

[2] Meyer and Eddie, *J. Lab. and Clin. Med.*, 1930, **15**, 447.

[3] Coleman, *J. Lab. and Clin. Med.*, 1930, **15**, 641.

[4] Mallardo, *J. Trop. Med.*, 1930, **33**, 125.

[5] Duncan and Whitby, "A System of Bacteriology," 1930, **5**, 396.

[6] Clark, *J. Bacteriol.*, 1917, **2**, 61.

All strains produced acid in excess of alkali in arabinose (final average pH 6.2), xylose (pH 6.6), and galactose (pH 6.8). Greater variations in the power to ferment were noted in the culture series containing glucose. Of 80 bovine strains tested 13 or 16% gave a final pH 6.2 or below, 28 or 33% had a final reaction of pH 7.3 or above. Practically every bovine strain fermented glucose to some degree. Five caprine strains recently isolated on the Island of Malta showed very little glucose utilization, while 3 strains isolated from Arizona goats produced sufficient acid to reduce the pH to 6.7. The average final reaction of 86 strains of human origin was pH 7.07 with 13% giving readings of pH 6.2 or less in glucose media. Porcine strains failed to utilize glucose in appreciable amounts, the final reaction averaging pH 7.5.

Levulose utilization almost paralleled that of glucose in the different groups. In general it was fermented slightly less than was glucose.

The fermentation of the monosaccharides can not be correlated in any way with the type of Brucella grouped either according to host origin, serologic tests, hydrogen sulfide production or genesistatic behavior.

5208

Lactate Production in Muscle Stimulated Briefly.

E. G. MARTIN, J. FIELD, II AND V. E. HALL.

From the Laboratory of Physiology, Stanford University.

In dogs anesthetized with amytal we have observed the lactate concentration in gracilis muscles immediately after brief periods of activity induced by short tetanizations repeated rhythmically. The muscles were stimulated *in situ* through the obturator nerve. The circulation was intact and the arterial pressure satisfactory. As soon as possible after stimulation ceased they were frozen *in situ* with carbon dioxide snow, excised and ground to powder in liquid air. Lactate determinations were made by the method of Friedmann, Cotonio and Shaffer.[1]

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[1] Friedmann, T. E., Cotonio, M., and Shaffer, P. A., *J. Biol. Chem.*, 1927, **73**, 335.

For comparison rectus femoris muscles of the same animals were excised in the same manner without previous stimulation and tested for lactates according to the same technique.

Results are as follows:

Stimulation period 30 seconds.

	Lactate in mgm.%		Lactate in mgm. %
Non-worked control	44	worked muscle	220
	56		165
Stimulation period 1 minute.			
Non-worked control	61	worked muscle	246
	63		264

The increases in lactate resulting from these brief periods of activity are equal to or greater than the increases reported by us after 15 minutes of tetanization under similar conditions.[2]

5209

The Therapeutic Use of Potassium in Certain Cardiac Arrhythmias.*

JOHN J. SAMPSON AND EVELYN M. ANDERSON. (Introduced by J. M. Read.)

From the Department of Medicine, University of California Medical School, and Mt. Zion Hospital, San Francisco, Calif.

The investigation of the action of potassium salts on the heart dates from the time of Ringer,[1] whose classical experiments on the frog heart are well known. Irrespective of osmotic tension, the necessity of sodium, potassium and calcium ions in a balanced solution was proved. Hering[2] stopped paroxysmal ventricular tachycardia and ventricular fibrillation in the dog, and many investigators including Rothberger and Winterberg,[3] Anrep,[4] and Wiggers[5] have similarly obtained immediate cessation of both auricular and ventricular ectopic rhythms by intravenous or intracardiac injections of solutions of potassium chloride. Wiggers[5] suggested the possibility of its therapeutic use in ventricular fibrillation of accidental electrocution. It thus seemed reasonable to attempt to disturb the calcium-potassium ratio in favor of potassium for certain other therapeutic reasons. Scherf[6] and others have demonstrated a reversal of the customary actions of potassium and calcium when used intravenously on ventricular ectopic rhythms caused by aconite. The calcium eliminated the aberrant beats, while the potassium precipitated showers or paroxysms of them. It is predicted that some cases may be encountered clinically in which the arrhythmias show such a paradoxical response to potassium.

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[2] Martin, E. G., Field, J., II, and Hall, V. E., Proc. Soc. Exp. Biol. and Med., 1929, **26**, 292.

* We are indebted to Dr. J. M. Read and Dr. E. Kilgore for case histories, and to Dr. Wm. J. Kerr for the use of his clinical material, and for his helpful association. We wish to thank Dr. H. Becks for the serum potassium determinations.

[1] Ringer, S., *J. Physiol.*, 1882, **3**, 380; 1883, **4**, 429.

[2] Hering, H. E., *Zentralbl. d. Physiol.*, 1903, **17**, 1.

[3] Rothberger, C. J., and Winterberg, H., *Arch. f. d. ges. Physiol.*, 1911, **142**, 461.

[4] Anrep, A., personal communication.

[5] Wiggers, C. J., *Am. J. Physiol.*, 1930, **92**, 223.

Norn[7] showed an increment in blood potassium of 21% 40 minutes after the oral administration of 12 gm. of potassium chloride; and 61% increment in 2 hours. An appreciable increment was obtained in 30 minutes after 2 gm. of KCl in 2 of our cases.

Potassium salts were used clinically to check attacks of paroxysmal ectopic ventricular tachycardia, and to prevent the occurrence of auricular and ventricular ectopic beats. Four different soluble potassium salts were used: potassium chloride, potassium iodide, potassium citrate, and potassium acetate, all with apparently identical effect. It is presumed that the anion and acid-base influences are negligible. For later use, we have employed only the acetate, because it causes no gastric irritability when administered in raspberry syrup or similar menstruum.

It was found that potassium may be safely administered orally in doses of 1 to 5 gm. of certain soluble salts, to human cases of heart irregularities. In 12 cases of such oral administration in auricular or ventricular ectopic beats or tachycardias, definite control of the arrhythmias was obtained in 5 cases. In Case No. 1, of our series, two attacks of paroxysmal ventricular tachycardia, with duration of 8 hours and 4 days respectively, were checked within the period of expected absorption time for potassium. Of the remaining 7 cases, 4 were free of any other evidence of cardiac pathology; and of these 4, none showed even a suggestion of positive response to potassium. The failure to affect this group may be used to differentiate such patients from those with true myocardial damage. A low potassium content of myocardium is known to exist in cases of heart muscle failure, and may be the explanation for this difference in response. The influence of potassium on the arrhythmia occurred in 30 to 90 minutes after administration; and the necessary dose required varied in different individuals. The effect was maintained from 6 to 8 hours, and occasionally some effect was observed for the succeeding 24 to 48 hours. A definite effect on the electrocardiograms in certain cases after potassium administration was observed. Certain ill effects are occasionally noted, namely, gastric distress and diarrhea. The acetate and citrate caused less distress than the chloride or iodide. The paroxysmal nodal tachycardia observed in one case and the epistaxis and petechiae in another may have been due to potassium administration.

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[6] Scherf, D., *Z. f. d. ges. Med.*, 1929, **65**, 255.

[7] Norn, M., *Skand. Arch. f. Physiol.*, 1929, **55**, 213.

Conclusion. Potassium salt administration by mouth is effective in checking auricular and ventricular ectopic beats and tachycardia in the majority of cases of organic heart disease. The failure to affect arrhythmias in patients without other evidence of cardiac pathology may be used to differentiate this group from the former. Potassium administration does not prevent the occurrence of auricular fibrillation.

5210

The Relation Between Acidosis and Glucose Tolerance.

H. J. DEUEL, JR., AND MARGARET GULICK.

From the University of Southern California Medical School, Los Angeles.

In earlier work[1] it was shown that phlorhizin glycosuria the glucose tolerance was directly related to the nutritional condition of the animal. Low tolerance was noted in those animals which had been fasted and phlorhizinized for 5 to 6 days while the opposite was found in those dogs which had received large doses of carbohydrate 12 to 15 hours previous to the test, although these animals were still phlorhizinized. These variations were similar to those found in the same dogs in the non-phlorhizinized state after fasting or after feeding large amounts of carbohydrate before carrying out the glucose tolerance test.

In each case the increased tolerance is associated with an abolition or a considerable decrease in the ketosis. The present investigation was designed to determine whether the acidosis *per se* was the cause of the decreased ability to store carbohydrate. Since it is impossible to produce a ketosis and at the same time have a large carbohydrate storage, an acidosis was produced by the injection or oral administration of 10 to 15 gm. of NH_4Cl. Simultaneously with the introduction of the NH_4Cl, large quantities of carbohydrate food were given. Glucose tolerance tests were made on the 2 following days using 16 gm. of glucose as was the practice in the earlier experiments with which these results are compared. The CO_2 combining power of the blood plasma was reduced from the normal value of 55 to 50 volumes percent to as low as 15 volumes percent in one case. Six tests were made on 3 dogs and these all showed a very small rise in the blood sugar with a rapid return to the preglucose level. These results are identical with those obtained on the same animals in the well fed condition whether normal or phlorhizinized. It is concluded that acidosis is not a primary cause of decreased glucose tolerance.

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[1] Deuel, H. J., Jr., *J. Biol. Chem.*, 1930, in press.

5211

Does Copper Poisoning Produce Pigmentation and Cirrhosis of the Liver?

ERNEST M. HALL AND EATON M. MAC KAY.

From the Department of Pathology, University of Southern California School of Medicine, Los Angeles, and from Scripps Metabolic Clinic, LaJolla, California.

Mallory, Parker and Nye[1] demonstrated pigmentation and cirrhosis of the liver in rabbits following administration of copper salts in their feed. Hall and Butt[2] repeated and extended the work of Mallory. They obtained a similar pigmentation and cirrhosis of the liver in rabbits by feeding copper acetate. In addition they made chemical analyses of the livers and demonstrated the presence of large quantities of copper. Flinn and VonGlahn[3] repeated many of Mallory's experiments and added others of their own. They concluded that copper salts and powdered metallic copper administered in the feed do not cause deposits of pigment in the livers of rabbits, rats and guinea pigs; nor do these substances produce cirrhosis. They maintained that animals fed on an exclusive diet of carrots developed a pigmentation comparable to that produced by Mallory and others. Polson,[4] in Great Britain, obtained results substantiating those of Flinn and VonGlahn.

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[1] Mallory, F. B., Parker, Frederic, Jr., and Nye, R. N., *J. Med. Res.*, 1921, **42**, 461.

[2] Hall, E. M., and Butt, E. M., *Arch. Path.*, 1928, **6**, 1.

[3] Flinn, F. B., and VonGlahn, W. C., *J. Exp. Med.*, 1929, **49**, 5.

[4] Polson, C. J., *Brit. J. Exp. Path.*, 1929, **10**, 241.

In view of these conflicting results it has seemed necessary to examine this problem again by a series of well controlled and carefully conducted experiments.

Methods. Healthy rabbits were selected. For each rabbit to be fed on copper, a litter mate of the same sex was selected as a control. Each series when 85 days old was placed on a special diet consisting of a mixture of ground alfalfa and ground barley. When 90 days of age, one series was placed on a diet in which 2 mg. of copper were added to each gram of the special diet. These diets and tap water were allowed *ad libitum.* Many of the copper fed rabbits died after several weeks due to copper poisoning, while none of the control rabbits died before being sacrificed.

Results. Seventeen out of 21 copper-fed rabbits showed pigmentation of the liver. The pigmentation consisted mainly of hemofuscin granules which were found closely packed in large Kupffer multinuclear giant cells. These large cells were found most abundantly about the portal spaces but many were distributed through the lobules as well. A few of the livers contained hemosiderin in small amounts. Ten out of 21 rabbits showed cirrhosis of the liver. When we consider that 5 of the 21 rabbits were fed copper for less than 30 days, it is evident that the percentage of pigmentation and cirrhosis is relatively high in those animals fed for 30 days and more. In the control rabbits practically no copper was found in their livers, while in the copper-fed animals there was found from 9.68 mg. to 237 mg. of copper per 100 gm. of wet liver tissue. The copper-fed animals showing no pigmentation or cirrhosis were animals showing less than 100 mg. of copper per 100 gm. of liver substance. All but one animal that received copper for more than 40 days showed pigmentation, which in most cases was very marked. All of the animals receiving copper for more than 60 days showed cirrhosis of the liver. The animals were fed on their special diets for periods ranging from 21 to 105 days.

The question "does copper poisoning produce pigmentation and cirrhosis of the liver" must, it appears, be answered in the affirmative at least for the experimental animals employed.

New York Section.

New York Academy of Medicine, November 19, 1930.

5212

The Pharmacology of Inflammation. I. Technic.

LOUIS HIRSCHHORN AND M. G. MULINOS.

From the Department of Pharmacology, College of Physicians and Surgeons, Columbia University.

In the induction of inflammatory reactions, two desiderata should be satisfied: (1) The reaction produced must be readable and fairly quantitative. (2) It should be repeatable a number of times in the same animal. For this purpose the conjunctival mucus membrane of the rabbit offers a sensitive surface well supplied with nerves and blood vessels; easily observed without undue disturbance to the animal, and anatomically constructed into a pouch capable of holding drugs for a relatively long time. The experiments of Amberg, Loevenhart, and McClure,[1] and Chiari and Januschke[2] show conclusively that pure mustard oil so injures the conjunctival mucus membrane and the cornea that only one experiment can be performed on any individual animal. It is obvious that a technic which would not produce a permanent damage to the structure of the eye would permit a more reliable study of the effects of drugs on the inflammatory process.

In the experiments here reported, the objections to the use of pure mustard oil as the irritant were obviated by substitution of a 15% solution of essential oil of mustard in mineral oil. One drop of the latter instilled into the conjunctival sac of a rabbit produced a reaction which was readable, and from which the animal recovered completely in from 7 to 14 days, at which time the experiment could be repeated. Some of the animals were used for as many as 14 experiments, without permanent injury to the eye. In 114 instillations made in 27 rabbits, the following phenomena were constantly observed: (a) blepharospasm which lasted about an hour; (b) lachrymation for about 15 minutes; (c) immediate injection of the conjunctival blood vessels, which reached a maximum in 15 minutes, but was still present after 48 hours; (d) pin-point miosis, the pupil attaining the size of the control pupil in about one hour; (the miosis was unaffected by atropine); (e) edema of the conjunctival mucus membrane, which constituted the most constant and readable phenomenon of the inflammatory reaction. The edema was first observed 5 minutes after the instillation, and reached a maximum in one half hour, at which time the sac-like swelling of the lids caused their palpebral margins to bulge from the globe of the eye. The reaction was read as "4 plus" only when all 4 components of the conjunctival mucus membrane—namely, upper and lower lids, nictitating membrane, and scleral portion, were maximally involved. If only 3 of these components were involved, the reaction was marked "3 plus". A total absence of edema was recorded as "no reaction", regardless of the appearance of any of the other phenomena enumerated above.

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[1] Amberg, Loevenhart, McClure, *J. Pharm.*, 1917, **10**, 209.

[2] Chiari and Januschke, *Arch. f. exp. Path. u. Pharm.*, 1911, **65**.

It now seemed desirable to try to induce this inflammatory reaction in animals injected with drugs known to inhibit inflammatory processes. Sodium salicylate and quinine bisulphate were the drugs chosen.[3, 4] Nine rabbits were injected intravenously with a 10% solution of sodium salicylate, a total of 17 times, the dose each time being 300 mg. per kilo of animal. Ten rabbits were injected intravenously with 1% solution of quinine bisulphate a total of 22 times, the dose each time being 30 mg. per kilo of animal. The number of "inhibition" experiments per animal was limited by the deleterious effects of the dose of drug necessary for the production of complete inhibition; the factor of safety is small, for the dose necessary to inhibit inflammation is very close to the minimal lethal dose.

The technic employed in these experiments was as follows: (a) One drop of 15% mustard oil in mineral oil was instilled into one conjunctival sac of a rabbit. The animal was observed until the edema reached a maximum (one half hour), (b) inhibitor drug was then injected intravenously, (c) within 5 minutes, one drop of irritant was instilled into the conjunctival sac of the other eye.

This technic permitted a direct comparison between inflammatory reactions produced on the same day, as well as a comparison of the reactions produced from day to day. It also furnished data as to any possible "curative" effect that inhibitory drug might have on the

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[3] Januschke, *Weiner Klin. Woch.*, 1913, **26**.

[4] Yasko, Ikeda, *J. Pharm.*, 1916, **8**, 137.

"normal control" reaction in the eye first instilled. In 39 experiments, "curative" effect was observed only once.

The appended table enumerates the results obtained in 75 control experiments, as compared with 17 experiments in which sodium salicylate was administered, and 22 experiments in which quinine bisulphate was used. It also furnishes an adequate indication of the readability of the edematous reaction, and the faithfulness with which it may be reproduced. This method is being used to investigate a number of other drugs.

TABLE I.
The incidence of edema elicited by 15% mustard oil in mineral oil, and its suppression by sodium salicylate and quinine bisulphate.

Reaction	Controls (75)	Sodium Salicylate (17)	Quinine Bisulphate (22)
(++++) max.	55	0	0
(+++)	15	0	0
(++)	5	5	1
(+)	0	5	10
No reaction	0	7	11

5213

Protein Sulfhydryl Groups.

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The importance of the sulfhydryl (SH) groups of proteins in cellular respiration has been clearly recognized (deRey Pailhade,[1] Heffter[2]) and the appearance of these groups in some proteins as the result of denaturation has been demonstrated (Arnold[3]). For the study of both cellular respiration and protein chemistry, therefore, a means of measuring protein sulfhydryl groups is desirable. Investigation of them has depended mainly on the color reaction they give with nitroprusside. For this test the groups must be in the reduced (SH) and not in the oxidized (S-S) form. Heffter[2] supposed that since many denatured proteins, the denatured serum proteins for example, did not give a color with nitroprusside, they contained disulfide (S-S) rather than sulfhydryl (SH) groups. He was indeed able to reduce the disulfide groups of denatured serum proteins with sodium sulfite so that they then gave a color reaction with nitroprusside. Protein disulfide groups have also been reduced by means of zinc and hydrochloric acid (Arnold[3]), by soluble sulfhydryl compounds (Hopkins[4]), and by cyanide (Walker[5]). On the other hand, protein sulfhydryl groups can be oxidized by sulfur (deRey Pailhade,[1] Heffter[2]), by disulfides (Hopkins[4]), and by other reagents. Since disulfide groups, as well as sulfhydryl, exist in proteins, and since these groups are interconvertible by oxidation and reduction, information will be incomplete until both the S-S and SH forms are measured.

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[1] deRey Pailhade, *Recherches Experimentales sur le philothion, etc.* Paris, Masson, 1891.

[2] Heffter, *Med. Naturwiss. Arch.*, 1908, **1**, 81.

[3] Arnold, *Z. Physiol. Chem.*, 1911, **70**, 300, 314.

First Method. We find that the color intensities given with the Folin-Marenzi[6] uric acid reagent by equivalent quantities of cysteine and cystine are precisely in the ratio of 2:1. This is contrary to what has been assumed (Folin and Looney[7]). If equal quantities of a denatured protein are completely reduced and completely oxidized and then hydrolyzed, the color intensities with the Folin-Marenzi reagent of the 2 hydrolysates are also in the ratio of 2:1. A sample of protein partly oxidized and partly reduced will yield a hydrolysate with a color intensity proportional to its state of oxidation-reduction. Measurements can be made to within 5-10%.

Second Method. The procedure is based on the fact that protein SH groups are oxidized by a suitable disulfide, the S-S groups of the latter thereby being converted into SH groups which can readily be estimated. The validity of this method depends upon proof that it is only SH groups of protein that react with a disulfide, that the reaction is in fact specific. Evidence of specificity has been secured by showing that in a number of completely reduced denatured proteins that number of SH groups measured is equal to twice the number of cystine molecules obtained on hydrolysis.

The 2 methods outlined yield results agreeing to within less than 10%.

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[4] Hopkins, *Biochem. J.*, 1925, **19**, 787.

[5] Walker, *Biochem. J.*, 1925, **19**, 1082.

[6] Folin and Marenzi, *J. Biol. Chem.*, 1929, **83**, 109.

[7] Folin and Looney, *J. Biol. Chem.*, 1922, **51**, 428.

5214

Experimental Studies of Amyloidosis.

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(Introduced by Benjamin Kramer.)

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Amyloidosis in humans is a secondary condition, resulting from a long continued suppurative infection or wasting disease. It is believed by some that it is not a degenerative process, but a physicochemical disorder. The amyloid substance is deposited in the organs, initiating changes which interfere with the function of the organ. The theoretical aspects of this question will be discussed in a subsequent paper.

Some favorable clinical results obtained in a group of patients suffering with amyloidosis secondary to bone tuberculosis, as a result of administering a concentrated powdered whole liver preparation, led us to study this matter experimentally. White mice were used as the experimental animals.

The mice were set into 3 groups. Thirty mice of Group I received diet No. 1, which consisted of oats, powdered whole milk, bread and water. Forty mice of Group II were fed diet No. 2, which contained chopped raw meat in addition to the constituents of diet No. 1. Diet No. 3, which included the liver preparation* plus diet No. 1, was given to the 40 mice of Group III.

Amyloidosis was produced in the white mice by subcutaneous or intramuscular injections of a 5% aqueous suspension of sodium caseinate (Pfannstiehl) which is also designated as nutrose solution. The inoculations were given daily for 6 days, omitted on the seventh day. This procedure was repeated weekly. The amounts given varied from 0.3 cc. to 0.5 cc. per day.

To detect the earliest possible traces of amyloid formation, differential staining, intra-vitam with 1% Congo Red was used. This was in accordance with the methods of Smetana[1] and Jaffe[2], and stained only the amyloid.

The following procedure was used: Under ether anesthesia, the thoracic bony wall was exposed but not opened, and 0.5 cc. to 1.0 cc. of a 1% aqueous solution of Congo Red was injected directly into the heart. The fluid was allowed to run into the heart without extraneous pressure. The mouse was sacrificed from 3 to 6 hours thereafter.

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* This liver preparation was generously supplied by Livermeal Corporation, Hoboken, N. J.

[1] Smetana, H., *Bull. Johns Hopkins Hosp.*, 1925, **37**, 383.

[2] Jaffe, R. H., *Arch. Path. and Lab. Med.*, 1926, **1**, 26.

The spleen, liver and kidneys were examined grossly. Blocks were hardened in Zenker's solution, imbedded in paraffin, cut, and the nuclei were stained with Delafield's haematoxylin. No cytoplasmic stain was used.

By this method, very early amyloid appeared as faint pink or violet intracellular inclusions, granular, homogeneous, or fibrillar in character. This was also observed by Smetana.[1] Adult amyloid appeared as a bright pink or red substance. In many instances, when stained according to Bennhold,[3] the microscopic findings were exactly as those seen in human amyloid. This amyloid, when in small amounts, appeared intracellular. In larger amounts, no cell structure could be detected, the amyloid appearing as homogeneous red masses surrounding vessels (as in the liver) or distending sinuses (as in the spleen), with a few shrunken, deeply pigmented and dense, or fragmented nuclei scattered within it. In no instance were we able to detect any cells or cell membranes separating the vessel lumen from the amyloid.

In the preliminary experiments, amyloidosis was produced successfully in the white mice after the 30th injection. Similarly in the present series of experiments, it occurred in the same period of time in Groups I and II. The mice in both groups showed essentially similar gross and microscopic changes at this stage and subsequently throughout the experiment. The first suggestive signs of amyloid formation in Group III appeared after the 58th injection. Definite evidence was found at the 66th injection. With continued inoculations, the amyloid changes became more marked in all groups. However, comparative studies (made at corresponding number of injections) showed strikingly less marked changes in Group III, definitely indicating a significant retardation in the development of amyloidosis.

Summary. 1. Amyloid first appeared in the spleen, then in the liver and later in the kidneys. In each instance this substance was first noted within cells of the reticulo-endothelial system. 2. Feeding concentrated powdered liver protects the animal against the formation of amyloid, delaying its formation markedly. In mice on ordinary laboratory diets or with chopped meat added to the diet, amyloid appeared after 30 injections of nutrose, in those kept on a laboratory diet plus powdered liver, no amyloid appeared until the

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[3] Bennhold, H., *Klin. Wchnschr.*, 1924, **3**, 1711.

58th injection, at which time the animals kept on the other diets showed amyloid grossly and in large amounts, as well as the secondary pressure effects of the amyloid.

5215

Influence of Age on the Effect of Parathormone on Guinea Pig Bones.

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We have already indicated the striking difference in the response of young and adult guinea pigs to relatively large single doses of parathormone.[1, 2] *For the purposes of this study we consider a guinea pig fully adult when the major epiphyseal cartilage plates of the humerus, tibia and femur are closed.*

We produced the essential lesions of ostitis fibrosa in young guinea pigs by daily administration of 10 to 20 units of parathormone for 10 to 34 days. It is usually assumed that a more prolonged period of treatment would produce a greater effect. However, the animals' increasing age during the period of treatment had to be considered. Four guinea pigs were treated daily, beginning at the age of 2 to 7 days, with daily doses increasing to 20 units during the last 2 to 3 months. The total length of the treatment was about 110 days, at the end of which time the epiphyses were not closed. In the treated animals the bones showed no marked changes when compared with their normal litter-mate controls, and the changes were insignificant when compared with those previously reported in younger animals. While the number of animals was small, the data were consistent enough to warrant the conclusion that the serum calcium is lowered toward the end of a long course of treatment of guinea pigs with parathormone.

We believe that for a time the severity of the lesions increased in these animals. However, when the animals reached a certain age the effect of parathormone on the bone was decreased, and healing actually occurred after that time in spite of the continued administration of parathormone. This is not to be interpreted as evidence of immunity developed in the course of the treatment.

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[1] Bodansky, A., Blair, J. E., Jaffe, H. L., *J. Biol. Chem.*, 1930, **88**, 629.

[2] Jaffe, H. L., Bodansky, A., Blair, J. E., *Arch. Path.*, in press.

Three guinea pigs about 3 weeks old were injected increasing doses (60 to 140 units) of parathormone at intervals of 7 to 11 days, after a preliminary short period on smaller doses. Two guinea pigs killed at the age of about 145 days (5 days after the last dose), showed healing fractures at the costochondral junctions indicating effects of only the last previous dose. An interval of 10 days between the doses seems sufficient to allow for almost complete healing. One guinea pig died at the age of 96 days, (48 hours after the last dose). The animal being younger, the bone changes were more definite, and the effects of other previous doses probably still persisted.

We tested the limits of resistance of adult guinea pigs to parathormone, in view of our earlier findings of absence of bone changes after single doses of 20 units per 100 gm. A 900 gm. guinea pig received 900 units of parathormone, and a guinea pig weighing 1040 gm. received 2080 units. The first animal was killed 48 hours after the injection, at which time it was apparently well; the serum calcium was 15.7 mg., but serum phosphorus and blood urea nitrogen were normal. The second died 40 hours after the injection. *In contrast to our results with young animals, it was shown that enormous and fatal single doses may be given to adult guinea pigs without producing bone lesions.*

We administered to an 860 gm. guinea pig 2580 units of parathormone in 3 equal doses pyramided within 48 hours. Urgent symptoms were absent 24 hours after the last injection, with a serum calcium of 19.6, and a serum phosphorus of 4.7 mg. per 100 cc. But 24 hours later urgent symptoms developed, associated with a serum calcium of 18.9, a serum phosphorus of 8.4 and a urea nitrogen of 40 mg. per 100 cc. The epiphyseal cartilage plates in this animal were not fully closed, as the cartilage cells were still arranged in columns. It is therefore all the more significant that only very slight bone resorption occurred in this animal. The evidence of bone resorption consisted chiefly in the appearance of numerous Howship's lacunae containing osteoclasts, especially beneath the endosteum.

Our acknowledgement is due to Eli Lilly and Company for supplying the parathormone used in these experiments.

5216

Attempts at Chemotherapy in Experimental Poliomyelitis.*

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The experiments to be briefly presented deal with a systematic investigation into the merits of a number of pharmacological substances as possible chemotherapeutic agents in experimental poliomyelitis in the monkey. The list of substances studied includes the following chemotherapeutic compounds, disinfectants, and drugs: silversalvarsan, neosalvarsan, tryparsamide, colloidal bismuth, bismuth potassium tartrate, triphal (organic gold compound),† methylene blue silver, antimony potassium tartrate, neostibosan (organic antimony compound),† mercurochrome, metaphen, plasmochin,† Bayer 205 (Germanin),† chaulmoogra oil esther,† and hexylresorcinol solution. Since it soon became evident that restraining an already established infection was a hopeless task, we have for the most part administered the above mentioned substances during the incubation period by intravenous, intramuscular, or occasionally intraspinous injection of maximal doses, repeated as a rule at intervals of 2 or 3 days. The experiments were set up in small groups, comprising in each instance 4 or 5 treated animals and one, 2, or more untreated controls. The infection was produced by intracerebral inoculation with 1 cc. of the supernatant of a centrifuged 5% or 10% virus cord emulsion (Aycock strain). Although we have noticed at times slight fluctuations in virulence, as indicated by variation in the length of the incubation period and by the extent of the infectious process, this strain, during a period of 1 year, has never failed to bring down every normal control monkey after intracerebral infection with the above dosage. Needless to say, the authenticity of the lesions was verified in each case by histological examination of the cord. The remarkable uniformity of the infection may, in part, have been due to our careful selection of passage virus, which was obtained by pooling cord sections from different levels of at least three or four different poliomyelitic monkeys, killed at the height of the infectious process. The experimental data col-

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* Under a grant from the International Committee for the study of infantile paralysis, whose work is being financed by Jeremiah Milbank.

† These substances were received through the courtesy of the Winthrop Chemical Company of New York City.

lected in the present study are based on observations bearing on 74 different monkeys, of which 42 were treated animals and 32 untreated controls.

As regards the prophylactic value of the various substances enumerated above, a certain antagonistic effect on the infection was observed particularly with the antimony compounds and the chaulmoogra oil esther, when administered repeatedly during the incubation period. Out of 11 antimony-treated monkeys 3 remained entirely free from symptoms, one showed an abortive form of the disease (transitory slight paresis of one arm only) and 2 developed much delayed paralysis, while in the remaining 5 animals the course of the acute infection, for all practical purposes, was fully as severe as in the accompanying controls. Of a total of 4 animals receiving chaulmoogra oil, 2 escaped the infection entirely, the other 2 developed partial paralysis. A certain prophylactic effect was noted after the administration of hexylresorcinol, particularly if the drug was given by the intraspinal route; of a total of 5 monkeys treated with this substance, one remained entirely free, one showed an abortive infection, while 3 succumbed to the disease. Neither antimony, nor hexylresorcinol solutions were viricidal *in vitro*. The next promising drug perhaps was mercurochrome, 2 monkeys after treatment developing only partial paralysis with very rapid convalescence and almost complete recovery. None of the other chemicals displayed the slightest effect whatsoever on the course of the infection. Reinoculation of those monkeys which had escaped the first infection presumably on account of the chemotherapeutic treatment demonstrated that these animals were fully susceptible.

In evaluating the above data, it should be borne in mind that the untreated infection in the controls ran not only a morbidity of 100% but also a very high mortality.

The small number of experimental animals precludes the drawing of any stringent conclusions, although the results obtained particularly with the antimony compounds and chaulmoogra oil may raise hopes for the possibility of influencing the disease favorably. The work is being continued with these two substances in an effort to obtain a more uniform chemotherapeutic effect.

5217

Effect on Costal Respiratory Movements of Division of the Phrenics Following Transection of the Midbrain.*

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It has been shown that section of the dorsal roots of the thoracic nerves is followed by cessation of costal respiratory movements which are promptly resumed if the phrenics are excised, the vagi and midbrain being intact.[1] (The effect of section of the dorsal roots is somewhat greater when the cervical as well as the thoracic roots are sectioned.[2])

Section of the midbrain alone at the level of the inferior colliculi is followed by a diminution of costal movements and the rate of respiration is somewhat slowed, but remains regular.

The question arises therefore whether section of the phrenics would have the same effect on costal respiration after section of the midbrain as after section of the dorsal roots of the thoracic nerves. The observed effects of division of the phrenics subsequent to transection of the midbrain in 30 cats are:

1. An increase in the depth of the costal respiratory movements, which is to be expected.

2. The respiratory rhythm immediately becomes irregular. There may be pauses of 5 to 10 seconds followed by from 5 to 10 costal movements and a succeeding pause. In some cases the tendency to grouping is slight. The character of the individual movements may vary. Sometimes there may be a rapid abrupt inspiratory movement followed without pause by an equally abrupt expiratory movement with a rhythm of 10 to 12 a minute. A series of from one to 3 small inspiratory movements may precede the actual large abrupt inspiration. When the group character is more pronounced, the inspiration is not so abrupt and expiration follows after a more appreciable interval.

So long as the vagi are intact, section of the dorsal roots of the phrenics, following transection of the midbrain does not elicit irregular respiration. Irregularity appearing after division of the phrenics is not due merely to elimination of afferent impulses. It would

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* The cost of animals for this study was defrayed by a grant from the American Association for the Advancement of Science.

[1] Pike, F. H., and Coombs, H. C., *Science*, 1922, **56**, 691.

[2] Coombs, H. C., PROC. SOC. EXP. BIOL. AND MED., 1929, **27**, 196.

FIG. 1.
The upper tracing is of costal, the lower, of diaphragmatic, respiration. The figure shows respiration after division of the posterior *corpora quadrigemina*, followed by excision of both phrenics.

appear that the vagus which under the conditions of the experiments here recorded is the only afferent mechanism from the respiratory tract and muscles to remain uninjured, has a closer relationship to the control of diaphragmatic movements than it has to the control of costal movements.

5218

Total Erythrocyte and Leucocyte Counts in Pregnant and Non-Pregnant Albino Rats.

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Crosman[1] recently stated that the average leucocyte count of the pregnant albino rat is approximately 7,000 to 10,000 higher than that of non-pregnant animals. Comparison with our data on pregnant and non-pregnant rats respectively, shows that there is such great fluctuation in the counts of individual rats that only averages from a long series are significant in this regard. Our average count for the leucocytes of the pregnant female rat throughout the last third of pregnancy (16 to 22 days) is 10,758 per cu. mm. of blood as against 12,372 for our non-pregnant animals. (Table I.) These results suggest a decrease in the average count of the leucocytes during the last third of pregnancy. Certainly no leucocyte increase occurs. A high peak appears in one maternal count at the time when the fetuses are from 23 to 27 mm. long (19th day), namely, 16,000 cells. The fact that 3 other rats with fetuses at this same stage of development showed a similar increase arrests attention. However, we do not consider it justifiable to conclude that there is a significant increase of leucocytes during pregnancy.

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[1] Crosman, A. M., *Anat. Rec.*, 1930, **45**, 259.

TABLE I.

Table showing the average erythrocyte and leucocyte counts of pregnant and non-pregnant albino rats. σ is the standard deviation for the leucocyte counts.

	No.	Average Erythrocytes	Average Leucocytes
Non-pregnant females	28	9,336,071	12,372 σ3,541
Pregnant females	29	8,086,551	
	9		10,758 σ2,583

To further check our results we have compared them with the average leucocyte counts for non-pregnant rats given by Adams and Shevket[2] from Wistar stock and from Mt. Holyoke stock respectively. In the Wistar stock these investigators report an average leucocyte count of 12,540 per cu. mm.; and in the Mt. Holyoke stock 10,750. When compared with these averages, our counts show no significant discrepancy.

The erythrocyte counts from pregnant and non-pregnant rats show such a slight difference that we conclude that there is no change in the average count during pregnancy. The average erythrocyte count for 28 non-pregnant females is 9,336,071 per cu. mm.; and for 29 pregnant animals 8,086,551. The highest erythrocyte count occurs simultaneously with the highest leucocyte count in pregnant rats, *viz.*, when the fetuses are at the 23 mm. stage of development.

TABLE II.

Table showing the extremes of variability in the number of red and white blood corpuscles per cu. mm., respectively in pregnant and non-pregnant albino rats.

	Highest rbc count	Lowest rbc count	Highest wbc count	Lowest wbc count
Pregnant	10,660,000	6,100,000	16,000	8,200
Non-pregnant	12,990,000	6,970,000	20,000	8,500

The variability of both rbc and wbc counts is greatest in the non-pregnant rats. In all groups the corpuscles are more numerous in the non-pregnant animals. Examination of the counts in Table II alone would lead one to suspect conditions of slight anemia and leukopenia as being characteristic of pregnancy in the rat. However, the differences which appear in the averages as given in Table I do not seem sufficient to us to draw such a conclusion, because of the error inherent in the technical methods employed in making blood counts.

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[2] Adams, E. A., and Shevket, F., *Physiol. Zool.*, 1929, **2**, 181.

5219

Hepatectomy in the Salamander with Special Reference to Hemopoiesis and Cytology of the Liver Remnant.

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In the salamander, *Triturus viridescens,* granulocytopoiesis is normally restricted almost completely to the subcapsular region of the liver, erythrocytopoiesis and thrombocytopoiesis to the spleen. A relatively very slight granulocytopoietic activity occurs in the intestinal mucosa. As previously reported, splenectomy does not effect a compensatory erythrocytopoietic or thrombocytopoietic activity in the lympho-granulocytopoietic envelope of the liver. Following splenectomy the processes of erythrocyte and thrombocyte production are shifted to the general circulation.[1] The reverse experiment also gives a negative result; hepatectomy effects no compensatory granulocytopoietic activity in the spleen. Since the same ancestral cell, the lymphoid hemoblast, occurs in both regions, as a mesenchymal derivative, it must be concluded that certain specific conditions in spleen and liver are restrictive as regards hemocytopoietic stimuli. The differential stimuli for erythrocyte and granulocyte formation presumably inhere in some element in the character of the blood supply; the perihepatic granulocytopoietic region contains a very meager vascularization as compared with the abundant sinusoidal venous system of the spleen.

Total hepatectomy results in death within 9 days (20 specimens). The survival time is uninfluenced by coincident splenectomy. Removal of half of the liver (left and middle lobes) produces no noticeable hemopoietic effects (30 specimens). Such experimental animals lived until killed during the third month for study of the remaining liver tissue. These one-lobe livers show no microscopic evidence of regeneration. The remaining lobe, however, is somewhat enlarged by the twenty-seventh day, and has apparently

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[1] Jordan, H. E., and Speidel, C. C., *Am. J. Anat.*, 1930, **46**.

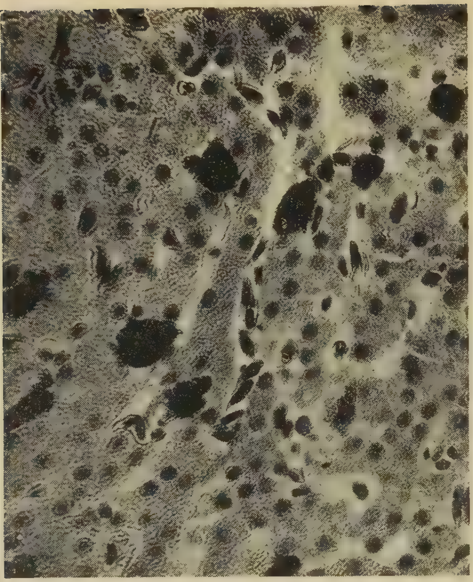

FIG. 1.

Photomicrograph of small typical area of liver of salamander 36 days after the removal of the complete left lobe. The histology is essentially normal. The black irregularly spheroidal areas are collections of pigmented macrophages lodged in sinusoids. The pigment is a derivative of disintegrated erythrocytes. Magnification 300 diameters. Helly fixation, eosin-azure stain.

effected satisfactory histologic and physiologic restoration (Fig. 1). The effect of semi-hepatectomy appears to be one of compensatory hypertrophy. Since no proliferative activity was observed at the several progressive stages studied the exact method of this hypertrophy remains uncertain. The enlargement is due in part to a thickening of the granulocytopoietic capsule, where very active mitosis occurs apparently in compensation for the loss of the excised portion. Individuals splenectomized at the time of removal of half of the liver (15 specimens), without marked injury to the blood supply or bile duct, also remained alive and apparently normal for over 2 months.

Removal of variable but relatively small apical portions of the left hepatic lobe produces no noticeable hyperplastic regeneration (30 specimens). At the end of 24 hours the cut surface is generally covered by a blood clot. Immediately beneath the clot for a variable depth there occurs a variable degree of histolysis of hepatic cells accompanied by a shrinkage of the entire region. Between the second and sixth days a peritoneal membrane forms beneath the thrombus and completely covers the reorganized parenchyma. By the ninth day this regenerated portion of the capsule has become slightly thickened by the addition of fibrous connective tissue. No subcapsular granulocytopoietic tissue has appeared in the region of section by the end of the tenth day.

Excision of more than one-half of the liver produces marked alterations in the remnant (10 specimens). In these experiments there occurred absolutely no regeneration in the region of section, either from ducts or parenchyma. Within the healed-over remnant the hepatic cells enlarge and the parenchyma becomes loosened into irregular cords of cells separated by wide sinusoids (Fig. 2). The individual cells appear perfectly healthy, but the cytoplasm contains numerous vacuoles. The granulocytopoietic layer is greatly depleted. By the nineteenth day the cells have enlarged still more and the parenchyma has become largely dissociated (Fig. 3). The granulocytopoietic layer has disappeared except for an occasional cell. The liver cells still appear healthy, but stain less deeply blue with eosin-azure and the cytoplasm is more extensively vacuolated. The lobular remnants of this size are apparently incapable of effective restoration. This contrasts sharply with the results following the removal of large portions of the liver in the dog, as reported by

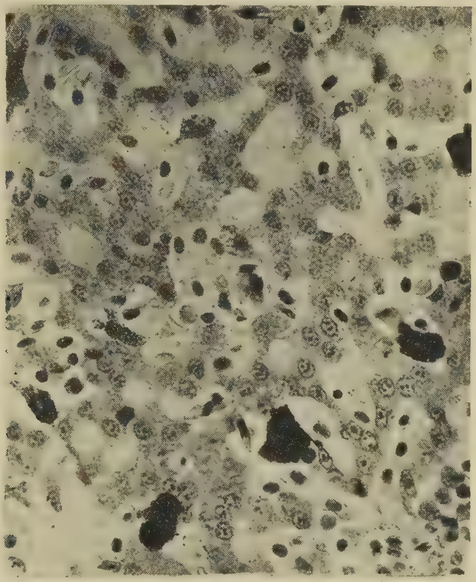

FIG. 2.

Photomicrograph of typical area of remnant of liver 6 days after removal of most of the liver and all of the spleen. Note the enlargement of the sinusoids and the coincident stretching and thinning of the cords, and the vacuolization of the cytoplasm of the constituent hepatic cells. Helly fixation, eosin-azure stain. Magnification 300 diameters.

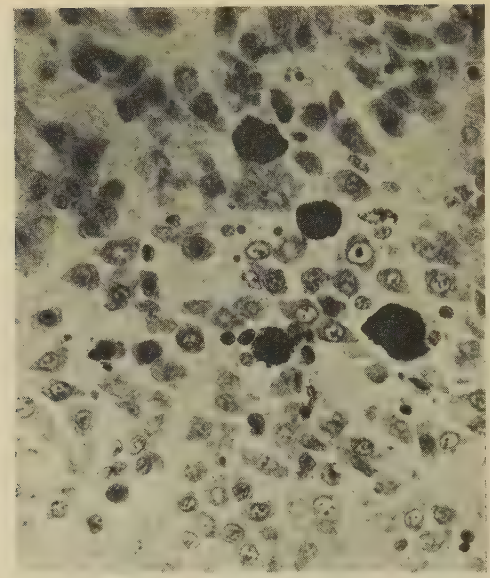

FIG. 3.

Photomicrograph of typical area of remnant of liver 19 days after removal of apical three-fourths. Note the complete dissociation of the parenchyma. Helly fixation, eosin-azure stain. Magnification 300 diameters.

Fishback.[2] Considering the relatively low evolutionary position of the salamander the apparent lack of regenerative capacity of the hepatic epithelium seems remarkable.

In this set of experiments the mortality was fairly high before the nineteenth day, but a few specimens lived several days beyond this time. Death was due no doubt in large measure to internal hemorrhage; in part to the anemia following an extensive disintegration of circulating erythrocytes. The regressive changes in the liver remnant must probably be attributed in large part to the disturbance of the blood supply.

The result of semi-hepatectomy depends upon the type of section. If the plane of division is sagittal, leaving a complete right or left lobe, a variable degree of compensatory hypertrophy takes place. When the section is made horizontally, separating distal halves of left, right and middle lobes, the same sequences of stages follow, ending in dissociation of the hepatic cords, as when only a small proximal portion is left.

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[2] Fishback, *Arch. Path.*, 1929, **7**.

5220

Menstruation and the Anterior Pituitary.

CARL G. HARTMAN, WARFIELD M. FIROR AND E. M. K. GEILING.

From the Carnegie Laboratory of Embryology and the Departments of Surgery and Pharmacology, Johns Hopkins Medical School.

The prevailing gynecological opinion in the textbooks and the gynecological literature generally holds that menstruation is due to the degeneration of the corpus luteum and that the menstrual flow proceeds exclusively from a "pregravid" (swollen, congested, secreting) endometrium. We contend that this is a *post hoc, propter hoc* argument, a time relation mistaken for cause and effect, a conclusion without the slightest experimental evidence.

The chief facts that have called this generally accepted notion in question were the outcome of the work of Geo. W. Corner, who found that periodic uterine bleeding in monkeys often does occur from uteri not at all enlarged and unaccompanied by corpora lutea in the ovaries. This conclusion has been corroborated by a very extensive series of observations involving scores of laparotomies and hundreds of rectal palpations of the organs in fully adult monkeys of the Carnegie colony at Baltimore.

On the basis of these findings and other considerations which will not be taken up at this place, the senior writer has contended[1] that there is an active substance originating outside the ovaries that causes the periodic bleeding which we call menstruation. Over a dozen hypophysectomies (Dr. Firor*) have enabled us to announce that it is the anterior pituitary that elaborates the substance causing uterine bleeding. The evidence is as follows:

1. Ablation of the hypophysis abolishes the cycle, the sex organs atrophy, the sex skin becomes pale as in the pre-adolescent female.

2. Administration of ovarian hormone (Allen-Doisy in the form of Amniotin Squibb†) to a female monkey, whether ovariectomized or not, causes the bleeding. Even a one-year-old baby, of the "equivalent age" of a 3- or 4-year-old girl, can be made to bleed

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[1] Hartman, C. G., *Am. J. Obst. and Gyn.*, 1930, **19**, 511.

* A description of the technique employed and the results of autopsies on the losses will appear in the *Contributions to Embryology*.

† These experiments were made possible through the kindness of E. R. Squibb and Son, through whose department of Biological Research the Amniotin and some of the alkaline extract of the anterior lobe was secured. Most of the anterior lobe extract was prepared by Dr. Geiling.

with as little as 90 rat units. The bleeding lasts for 10 to 15 days after the injections have stopped. This has been taken by other authors as a direct effect of the hormone on the uterus.

3. That the ovarian hormone acts through the hypophysis and not directly upon the genital tract is shown by the fact that if the animal is completely hypophysectomized the injections of Amniotin Squibb are without avail, that is, there is not the slightest bleeding whatever. In these experiments red blood cells are searched for in the vaginal lavage with the most meticulous care and may be found even when present in numbers that would absolutely escape notice by the ordinary smear method. Yet in one 1760-gm. baby hypophysectomized 3 weeks before and receiving 500 rat units of Amniotin Squibb over a period of 10 days not a single red blood cell was recoverable from the vaginal lavage. A control that had the brain retracted and compressed but the hypophysis left intact bled after receiving 160 rat units. These experiments establish beyond question a new function for the anterior lobe of the hypophysis. The experimental results are quite in line with clinical observations both before and after Fröhlich on the relation of menstrual anomalies and hypophyseal disturbances.

4. One adolescent female and one multipara gave a slight positive reaction to massive doses of Amniotin Squibb, a bleeding so meagre that it would have escaped notice by ordinary methods of examination. Presumably a small bit of the anterior lobe has been retained in these 2 animals. Tests for sensitivity to insulin bore out this assumption. The intensity of the menstrual flow may be more or less proportionate to the amount of active anterior lobe tissue.

5. Administration of anterior lobe hormone in any form—implants of fresh gland, or intraperitoneal injection of triturated gland tissue by trochar; or injection of acid or alkaline extract—all result in bleeding, whether the animal be normal, sick and amenorrheic, castrated or hypophysectomized, old or young.

6. The threshold for the bleeding phenomenon is immensely lower than that for growth and congestion of the uterus and ovaries. In most of the experiments no change in these organs took place; several times the Smith-Engle effect (stimulation of graafian follicles) took place along with the bleeding. Such difference points either to a separate hormone that conditions bleeding, or a mere difference in threshold of the affected organs. The fact that both acid and alkaline extracts bring about bleeding argues for the assumption of a distinct hormone.

7. Since bleeding is caused so much more readily than hyper-

plasia of refractory ovaries or a dormant uterus, the clinician must decide to what degree the patient is benefited whose amenorrhea is cured only to the extent of bleeding without a real awakening of the reproductive tract.

8. Upon these and similar facts a new theory of menstruation must be built. The sequence of events that occur in the ovulatory menstrual cycle, as determined by long and painstaking labor on the part of gynecologists during the last 100 years, will find a new explanation in the experiments here reviewed and menstruation will be placed upon an experimental footing.

5221

The Influence of Iodide of Iron on Rats Receiving a Vitamin A-Free Diet.

F. E. CHIDESTER, A. G. EATON AND N. K. SPEICHER.

From the Department of Zoology, West Virginia University.

In 1925, the senior author, who had been keenly interested in the endocrine glands since 1912, was struck by the fact that several medical writers had shown that thyroid extract was beneficial in cataract and certain other eye diseases involving keratization. Accordingly, he began experimenting with rats depleted of Vitamin A, using various iodin compounds.

Among the interesting discoveries was the fact that on a normal diet tiny quantities of iodin would induce greater growth. Subsequent studies carried on with various associates seemed to indicate quite definitely that xerophthalmia and other symptoms of Vitamin A deficiency would yield to iodin treatment, and the most effective combination proved to be with extremely small doses of ferrous iodide.[1]

Our studies of the efficiency of ferrous iodide have been carried on during 1929 and 1930, and we are convinced that the action of iodin is largely responsible for the prevention and cure of xerophthalmia, in our rats. But as the present findings indicate, the iodin-iron combination which is so effective in curing the major symptoms of Vitamin A deficiency, cannot replace the growth factor, since the iodin introduced is not balanced by unsaturated fatty acids such as are also present in cod liver oil.

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[1] Chidester, F. E., Eaton, A. G., and Thompson, G. P., *Science*, 1928, **68**, 432.

Thirty-six young rats, 30 days of age, and averaging about 35 gm. in weight, were placed on a diet deficient in Vitamin A and low in Vitamin D (Sherman No. 380), on January 24, 1930. Individual round (Sherman) cages were used, and distilled water was constantly before the animals. Weights were taken twice each week.

After xerophthalmia and constant or reduced weight had indicated depletion, the animals were divided into 6 lots, with split litters and corresponding weights, and all but the 2 sets of controls were furnished 1/100 mgm. of irradiated ergosterol daily, beginning April 4. The effects of the added Vitamin D lasted until about April 10, and on April 16 lots 1 and 2 received 5 drops of FeI_2, and lots 3 and 4 received 3 drops of FeI_2 in concentrations such that 5 drops daily furnished 0.0005 grain of I_2, and 0.000165 grain of Fe; while the 3-drop dosage furnished 0.0003 grain of I_2, and 0.000099 grain of Fe.

In lots 1 and 3 the animals were also irradiated for 30 seconds 6 days a week, beginning April 28.

Results. Lot 1 showed improved condition and increased growth from April 16 to 22, and accelerated growth from April 28 to May 6, but were evidently receiving too much iron and particularly too much *iodin,* for their depleted condition. Irradiation effect was marked.

Lot 2 showed stimulation with the FeI_2 and improved rapidly from April 28 for 2 weeks, but apparently needed additional irradiation stimulus to release stored foods.

Lot 3 furnished irradiated ergosterol on April 4, received 3 drops of FeI_2, and were themselves irradiated from April 28. They showed that dosage and irradiation were almost the optimum and on May 13 had reached the average weight of 125 gm., in comparison with the 118 grams of the controls. On June 3, 131 days after they were started on the experiment, 3 of the original animals were still alive, with an average weight of 136 gm., while the controls had reached the weight of 135 gm.

On July 1, two survivors of this lot still averaged 125 gm. in weight and exhibited no signs of xerophthalmia. The iodin effect was exercised in the absence of added fats, however, and growth was not resumed. Since the experiment had begun 158 days previously, and the iodide of iron had been furnished for 76 days, it seems evident that despite the absence of adequate fat, the iodin-iron combination was beneficial. Growth resumption could not be expected, however, since the iodin administered would tend to require unsaturated fats.

Lot 4 received ergosterol on April 4 and FeI_2 on April 16, exhibiting remarkable weight increase from the iodide of iron, and rising from an average of 62 gm. on April 16 to the high point of 85 gm. on May 2, but rapidly dying off. Apparently irradiation of the animals in lots 1 and 3 had a beneficial effect in aiding in the catalysis initiated by the iron-iodin. It is well known that the catalytic effect of ferrous iron is produced when it is oxidized to a ferric salt. Ferrous salts are not readily oxidized by iodin, but the digestive chlorine readily furnishes the oxidizer.

Lot 5 were controls receiving Sherman No. 380 and distilled water, and all of them died in April. In another paper, we shall show the probable effect had they also received ergosterol.

Lot 6 were controls receiving the deficiency diet until April 17 when they were also given 1 drop of Patch's Cod Liver Oil daily. The Cod Liver Oil group were completely protected against symptoms of Vitamin A deficiency, and grew steadily. It was not until June 6, however, that they began to show slight superiority (7 gm. average) to the animals in Lot 1, receiving ergosterol and ferrous iodide, with supplemental irradiation.

5222

The Nature and Importance of Surface Films on Chylomicrons.

S. DeW. LUDLUM, A. E. TAFT and R. L. NUGENT.
(Introduced by Stuart Mudd.)

From the Gladwyne Research Laboratory, Gladwyne, Pa., and the Department of Industrial Research, University of Pennsylvania.

It is generally recognized that blood transports free fat as an emulsion of minute droplets.[1, 2] Gage and Fish[1] in their classical study of the digestion, absorption and assimilation of fat, call these droplets chylomicrons, because they enter the blood with the chyle, the milky fluid containing absorbed food from the intestine which pours directly into the blood stream.

Blood serum containing chylomicrons forms an oil-in-water emulsion system[3] which may be called the chylomicron emulsion. Oil-in-water emulsions which may be as concentrated as the chylomicron emulsion always involve a third substance, the protective or emulsifying agent which is adsorbed in the oil-water interface. Protective films are thus formed around the disperse oil drops which prevent their coalescence, thus stabilizing the emulsion.[4] The authors are the first to study the protective films on the chylomicrons and to point out their importance in the transport of fat by the blood and their presumptive importance in its assimilation from the blood and its storage and final utilization.

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[1] Gage, S. H., and Fish, P. A., *Am. J. Anat.*, 1924, **34**, 1.

[2] Bloor, W. R., *Physiol. Rev.*, 1922, **2**, 92.

[3] Ludlum. S. DeW., Taft, A. E., and Nugent, R. L., *Colloid Symposium Annual*, 1929, **7**, 233.

The experiments have all been done with chylomicrons in human serum, but the extension of the results to human plasma and to the serum and plasma of animals should be a relatively simple matter.

Consideration of the known facts with regard to protective film formation and the composition of human serum indicates that the chylomicrons should be surrounded by protein films, very possibly consisting of serum albumin, serum globulin, or both. If they are surrounded by protein films their surface properties should be those of protein. Thus their isoelectric point should fall within the range characteristic of proteins,[5] very possibly within the range of pH 4.7—pH 5.4 characteristic of the ordinary serum proteins.

The pH of the isoelectric point of the chylomicrons in human serum has been determined both by maximum flocculation and cataphoretic methods. Both lead to values between pH 4.7 and pH 5.4, definitely within the range of the isoelectric points of the serum proteins. Six of 7 cataphoresis determinations gave values between pH 4.8 and pH 5.0. These results afford the best experimental evidence that can be brought to prove the correctness of the hypothesis that the chylomicrons are actually surrounded by protein films. If the chylomicrons possessed lipoid surfaces their isoelectric point would be much lower. With lecithin surfaces, for example, it would lie between pH 2 and pH 3.

The position of the isoelectric point between the isoelectric points of serum albumin and serum globulin, points to the interesting possibility that the protein films on chylomicrons consist of a mixture of the two.

The behavior of the chylomicrons when protein precipitants are added to sera containing them, and the behavior of isoelectric aggregates of chylomicrons when examined by means of the Mudd Interface Technique,[6] have also been shown to be in accord with the theory that the chylomicrons are surrounded by protein films and thus confirm the conclusion from the isoelectric point determinations.

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[4] Bancroft, W. D., "Applied Colloid Chemistry," McGraw-Hill Book Co., New York, 1926, 351.

[5] Abramson, H. A., Colloid Symposium Monograph, 1928, **6**, 128; *J. Gen. Physiol.*, 1929, **13**, 169.

These protein films around the chylomicrons determine the stability of the chylomicron emulsion and thus the very act of the transport of free fat by the blood.

If, as seems probable, cells take up chylomicrons as such in the assimilation of fat from the blood,[2] the surface properties are presumably of the greatest importance. It has been shown[7] that particles with lipoid surfaces (acid-fast bacteria) are not readily taken up by cells (phagocytized), whereas those with serum-protein surfaces (acid-fast bacteria treated with the proper serum) are readily taken up. The protein films on chylomicrons give them a protein surface which may well be the all-important factor in determining their direct assimilation from the blood by cells.

After assimilation chylomicron fat may be stored in the form of larger fat drops[2] or may be acted upon chemically in its final utilization by the body. Both must involve the destruction of the protein films, which must therefore be considered in the study of these problems. This destruction could readily be brought about by proteolytic enzymes.

5223

Hexylresorcinol in the Treatment of Hookworm Disease.*

P. D. LAMSON, H. W. BROWN, C. B. WARD AND B. H. ROBBINS.

From the Department of Pharmacology, Vanderbilt University School of Medicine.

Hexylresorcinol was recently found by us to be an effective ascaricide, killing *Ascaris suum* quickly *in vitro,* removing in single therapeutic doses practically 100% of the ascarids from dogs and 90% or more from man.[1, 2, 3] Leonard has found this substance

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[6] Mudd, S., and Mudd, E. B. H., *J. Exp. Med.*, 1924, **40**, 633, 647; Seifriz, W., in Alexander, J., "Colloid Chemistry," The Chemical Catalog Co., Inc., New York, 1928, **2**, 437.

[7] Mudd, S., Lucké, B., McCutcheon, M., and Strumia, M., Colloid Symposium Monograph, 1928, **6**, 131; *J. Exp. Med.*, 1930, **52**, 313; Strumia, M., Mudd, S., Mudd, E. B. H., Lucké, B., and McCutcheon, M., *J. Exp. Med.*, 1930, **52**, 299.

* The funds for carrying out this work were given by the International Health Division of the Rockefeller Foundation.

very non-toxic in his clinical work on urinary antisepsis,[4] and we have treated 1500 human cases for ascariasis without symptoms of any consequence.[5] Our laboratory experiments showed hexylresorcinol to be also effective against hookworm in dogs.[2]

Through the kindness of Dr. W. W. Cort of the Johns Hopkins School of Hygiene, and Dr. A. T. MacCormack, Commissioner of Health for the State of Kentucky, arrangements were made by which we could treat and re-examine cases of ascariasis studied in the survey being carried out by Dr. Cort on the distribution and incidence of ascariasis in the United States. A field laboratory was set up under the directorship of Dr. G. F. Otto of Dr. Cort's department, in Jackson, Kentucky, with a technical staff for making egg counts by the Stoll Method.[6] Dr. H. W. Brown of this department was in charge of all treatments, recollections, and recounts. Of the 6,000 cases examined by Dr. Cort's staff, 1,350 cases of ascariasis were treated by Dr. Brown and Dr. B. H. Robbins; 1,000 recollections were made 2 weeks after treatment, and egg counted. Among these, 530 cases harboring hookworms were found and treated. These hookworm treatments were incidental to the more complete studies of hexylresorcinol in ascariasis and are necessarily incomplete. The cases were treated with various amounts of the drug at different times in widely separated communities and under all manner of conditions. Those here reported are a summary of the effects of therapeutic doses of hexylresorcinol given as crystals in hard gelatin capsules and followed by water in some instances and mineral oil in others. In our laboratory experiments hexylresorcinol was found to be very effective when given with oil, but this is disagreeable to take, and in the field our ascaris studies showed that about as good results were obtained with water alone, which is a much simpler form of treatment.

The results are sufficient to show that hexylresorcinol given in crystalline form in hard gelatin capsules will remove 90% of the hookworms in a group of cases if precautions are taken to see that no food is allowed for some time before as well as after treatment.

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[1] Lamson, P. D., Ward, C. B., and Brown, H. W., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 1017.

[2] Lamson, P. D., Brown, H. W., and Ward, C. B., "Hexylresorcinol in Canine Ascariasis," in press.

[3] Lamson, P. D., Caldwell, E. L., Brown, H. W., and Ward, C. B., *Am. J. Hyg.*, 1931, March issue.

[4] Leonard, Veader, *J. Am. Med. Assn.*, 1924, **83**, 2005.

[5] Full report of 1500 cases to appear later.

[6] Stoll, Norman R., and Hausheer, Walter C., *Am. J. Hyg.*, 1926, **6**, 134.

TABLE I.
Hookworm treatment using hexylresorcinol.
Total egg counts (0.01 gm. formed basis) of all cases.

Amount of Hexylresorcinol	Followed by	Before Treatment	After Treatment	% Reduction	Total Cases	% Cases Negative after Treatment
1 gm.	Water	1003	198	80	24	41
1 "	30 cc. mineral oil	924	104	89	26	46
0.8 "	Water	3914	816	79	94	57
0.8 "	30 cc. mineral oil	264	61	77	20	60
0.6 "	Water	1681	237	86	21	43
Repeated Doses						
Two doses of 0.6 gm. given at intervals of 1 to 7 days. Total amount of hexylresorcinol taken, 1.2 gm.						
1.2 gm.	Water	774	118	85	52	63
1.2 "	30 cc. mineral oil	473	18	96	19	88

It has been found in all our work that the presence of food, especially of protein nature, greatly reduces the efficacy of hexylresorcinol as a vermicide. No symptoms of any consequence were observed when treatment was properly carried out. A few cases of nausea and vomiting occurred if food was taken shortly after treatment but the fact that whole schools were treated at a time, and no children ever refused or complained of re-treatment even on the day after the first dose shows, we believe, that the drug caused no serious disturbance. Whole communities refused treatment of any sort at first, believing that a vermifuge given several years previously was to be repeated.

We wish to express our thanks to those mentioned above, as well as to Dr. P. E. Blackerby and the many county health officers who made this work possible, and to Dr. Veader Leonard, of Baltimore, for his kindness in supplying us with the hexylresorcinol used in these treatments.

5224

The Use of Wires in Blood-Vessel Surgery.

WILLIAM M. MILLAR.* (Introduced by F. C. Lee.)

From the Surgical Hunterian Laboratory of the Johns Hopkins Medical School.

Experiments have been conducted to ascertain (1) what metal causes the greatest amount of blood clotting *in vivo*, and (2) to note the difference between and results in the so-called "wiring with electrolysis" and the non-electrolytic methods commonly employed in the treatment of aneurysms.

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* William S. Halsted Fellow in Surgery, The Johns Hopkins Medical School.

Ten different wires were used; namely, aluminum, bronze, copper, dental alloy, German silver, gold, piano wire, silver, pliable steel, and zinc. A small piece of each of these wires was inserted in the aorta of a cat and allowed to remain for 7 days. The animal was then killed and examined.

Zinc containing less than 0.1% lead, 0.01% iron, and 0.002% cadmium always produced clotting and was found to be the best metal for this purpose. Copper and its alloys produced clotting but to a much less degree, and steel and silver appeared to exert the least influence. Gold, aluminum, and platinum would seem to produce practically no clotting.

It was seen that the Moore-Corradi method as practiced surgically with a current of 50 ma. is apparently detrimental in both its immediate and its later effects to the strength of the vessel wall. Evidence points to the fact that such a procedure tends to weaken rather than to strengthen the vessel wall. The low current (10 to 12 ma. for 60 minutes) also causes some burning of the vessel wall. The amount of clotting produced by a low current of the strength commonly used in human surgery (10 to 12 ma. for 1 hour) is no greater than that produced by a similar wire placed without current in the freely running blood stream. The dangers of embolus formation are great.

All in all, however, it would seem that the wiring of aneurysms, although at all times a dangerous procedure, may be developed into a useful therapeutic measure.

5225

Effect of Histamine on the Secretion of Gastric Pepsin.

ALFRED GILMAN AND GEORGE R. COWGILL.

From the Laboratory of Physiological Chemistry, Yale University.

Histamine is known generally as a gastric secretogogue, and the results of many investigations have shown that the secretion of the normal stomach, in response to this stimulus, is rich in free acid; the response of the peptic glands, however, has received but slight attention. In a series of experiments upon human subjects, Polland

and Bloomfield[1] have observed that with an increased volume of gastric secretion in response to a histamine stimulation there is a progressive decrease in pepsin concentration, although the total amount of pepsin secreted is actually greater.

A new micro technique for estimating peptic activity was recently developed in this laboratory.[2] Utilizing this method in a series of experiments upon dogs possessing either Pavlov or Heidenhain pouches the authors find that:

(a) With increased volume of secretion produced by histamine stimulation there is a progressive decrease in the concentration of pepsin in the gastric juice.

(b) The pepsin concentration of the fasting juice is invariably greater than that of any subsequent sample.

(c) In the first sample of juice obtained following histamine stimulation the concentration of pepsin is diminished, but due to the increased volume of flow, the total amount of pepsin apparently secreted per unit of time is greatly increased.

(d) In subsequent samples the concentration of pepsin and the total amount of enzyme secreted per unit of time is rapidly diminished.

(e) Examination of samples collected subsequent to 45 minutes after the injection of histamine shows with few exceptions, that the total pepsin secreted per unit of time is less than is the case before the administration of histamine. The concentration of pepsin in these samples may be as low as 1/40 the concentration found in the fasting juice.

(f) The content of pepsin in the sample is roughly parallel to the content of neutral chloride.

The authors therefore conclude that histamine acts exclusively upon the acid secreting glands, merely filling the stomach with an acid fluid. The sudden rise in total pepsin immediately subsequent to the administration of histamine is in all probability due to a flushing of the lumen of the glands and the mucosa of the stomach with the acid secretion produced and in this way increasing the peptic content of the sample in a purely mechanical fashion. In support of this assertion the authors find that a second injection of histamine, made after the volume of the juice secreted in response to the first stimulation has begun to diminish, will not again bring about a sharp rise in total pepsin, the explanation being that the stomach has already been washed free of residual enzyme.

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[1] Polland, W. S., and Bloomfield, A. L., *J. Clin. Invest.*, 1929, **7**, 57.

[2] Gilman, A., and Cowgill, G. R., *J. Biol. Chem.*, 1930, **88**, 743.

These experiments were conducted upon 4 dogs having gastric pouches of the Pavlov type, and one having the Heidenhain pouch.

5226

The Toxicity and Balantidicidal Action of "Di-Hydranol" in Guinea Pigs.*

NORMAN A. DAVID AND CHAUNCEY D. LEAKE.

From the Pharmacological Laboratory of the University of California Medical School, San Francisco.

The frequent natural infestation of guinea pigs with such an easily observed intestinal parasite as *Balantidium coli* offers an excellent opportunity for a carefully controlled study of the parasiticidal action *in vivo* of new drugs of possible use in this regard. With proper toxicity studies of the same drugs in healthy uninfected guinea pigs, one may thus obtain quantitative data of considerable value in estimating the relative chemotherapeutic indices of a series of compounds, and thus the more readily estimate their worth for clinical trial in humans. Sweeney[1] has already nicely demonstrated the possibilities of this technique. We determined to use this method as an adjunct to our general investigation of the chemotherapy of amebiasis, especially to assist in determining whether or not a compound is worth the time and expense of study in natural or induced amebic infestation in monkeys or other animals.

Such studies of toxicity and experimental activity, together with quantitative data on the rate of secretion, we consider absolutely necessary before one may be justified in attempting the clinical evaluation of a new drug in human therapy.[2] New drugs are continually being offered for clinical evaluation in amebiasis and we are amazed that so many come to clinical trial without reference to quantitative data either on toxicity and therapeutic activity in experimental animals or rate of excretion in normal humans. Thus Faust has recently reported[3] on the clinical evaluation of 2-4 dihydroxy-phenyl-n-heptane ("di-hydranol") in humans before the publication of a proper evaluation of the relationship between the toxic and parasiticidal dosage of this compound as it may be estimated in experimental animals without involving danger to human patients. It is to supply this essential information that the present report is made.

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* This note is part of an extended cooperative study of the chemotherapy of amebiasis conducted by the Pacific Institute of Tropical Medicine of the Hooper Foundation for Medical Research and the Pharmacological Laboratory of the University of California Medical School, San Francisco, and supported in part by Eli Lilly and Co., Indianapolis, and the Ciba Co., Inc., New York City.

[1] Sweeney, M. A., *Am. J. Hyg.*, 1929, **9**, 544. We desire to express our thanks for advice from Dr. E. L. Walker and Miss M. A. Sweeney in connection with this study, and to Miss Dorothy A. Koch for aid in stool examinations.

[2] Leake, C. D., *J. Am. Med. Assn.*, 1929, **90**, 1632.

Crystalline "di-hydranol" was generously supplied by Dr. Veader Leonard. Its toxicity on single oral administration to guinea pigs was estimated by a general technique described previously,[4] with results as indicated in Table I. At the average lethal dose of 400 mgm. per kg. or higher, the guinea pigs lost markedly in weight, became lethargic, suffered from diarrhea, shivering and anorexia, and usually died within 4 days. On postmortem examination congestion of the small intestines, liver and kidneys was noted, and microscopic study of tissues obtained at such an examination revealed cloudy swelling of the kidney and liver. Some animals after a display of the symptoms above noted tended to recover somewhat, but a weakening of resistance was indicated by pneumonic sequelae terminating in death 10 days or more after the administration of the drug.

TABLE I.

Toxicity of "Di-hydranol" on single oral administration in guinea pigs.

Dose	Number used	Number died	Time of death
600 mgm./kg.	10	10	All died within 4 days of administration.
400 " "	10	5	3, 3, 3, 11, 14 days.
200 " "	5	0	—

In estimating the therapeutic value of "di-hydranol" in *Balantidium coli* infestations in guinea pigs, we applied the same rigorous criteria of "cure" as insisted upon by Sweeney.[1] We found that stools could be more readily secured in a satisfactory condition for examination by daily feeding of greens, about an hour after which individual stool samples were obtained by manual expression. Each animal was isolated in a separate cage with a wide mesh false bottom, and with food and water containers arranged to prevent contamination. The cages were cleaned and scalded daily. The animals selected for therapeutic study were heavily infested. Stools were examined daily during treatment and for 2 weeks after treatment ceased, when the "cured" animals were sacrificed in order to determine whether or not parasites might be found in the caecum or elsewhere in the intestinal tract. A control group of 5 heavily infested guinea pigs were followed for a 2 week period under the same conditions of hygiene as the treated animals. Treatment was continued in all pigs at the dosage indicated for 5 doses, whether the stools became negative or not, in order to estimate the possible danger of cumulative toxicity on continued administration of sublethal doses. The data presented in Table II indicate the wisdom of this procedure.

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[3] Faust, E. C., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 905.

[4] Anderson, H. H., and Leake, C. D., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 267; *Am. J. Trop. Med.*, 1930, **10**, 249.

TABLE II.

Curative power of "Di-hydranol" on repeated sublethal oral doses in *Balantidium coli* infestations in guinea pigs.

Dose mgm./kg.	No. of animals	No. cured	Total curative dose mgm./kg.	Time of cure days	No. not cured	Total dosage not curing mgm./kg.	No. died	Lethal dosage mgm./kg.
100 every 2 days	5	4	200	4	1*	100*	3	100, 200, 500
75 every 2 days	5	4	225	6	1	225	3	225, 300, 375
50 daily	5	2	150	4	3	1 at 150 2 at 250	1	accidentally at 150
No drug	5	0	—	—	5 at 14 days	—	0	—

* Died 20 hours after initial administration of drug; evidence of tuberculosis found at postmortem.

Summary. 2-4 dihydroxy-phenyl-n-heptane ("di-hydranol") causes death in guinea pigs in about 4 days with symptoms of loss of weight, diarrhea, and lethargy at an average dose of 400 mgm. per kg. on single oral administration. In natural *Balantidium coli* infestations in guinea pigs, it seems to be curative at a total of 200-225 mgm. per kg. on continued administration at daily or two daily intervals of 75 to 100 mgm. per kg. Toxicity may be encountered, however, at a total dosage only slightly higher than this indicated curative range on continued administration of the sublethal doses proposed.

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Ammonium Metavanadate as a Reagent for Reducing Carbohydrates.

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A reagent consisting of 2% ammonium metavanadate in 10% sodium carbonate solution has proved effective in the detection of reducing carbohydrates or of non-reducing carbohydrates after hydrolysis. To make a test we heat on the water-bath 2 cc. of a 0.25% or 0.50% solution of the compound under consideration with 5 cc. of the reagent. A brownish to yellow color develops with a slight precipitate. On the addition of 1% hydrochloric acid, the mixture becomes clear and assumes a characteristic green color, due to the reduction of the metavanadate to a compound in which vanadium holds a lower valence. The acid must be added in quantities just sufficient to neutralize the reaction mixture, the point of neutralization being determined with litmus paper.

The following compounds reduced with the formation of the characteristic color: pentoses (xylose, arabinose, rhamnose); hexoses (glucose, glucosamine, levulose, galactose, mannose); disaccharides (lactose, maltose, cellobiose). The following compounds reduced only after preliminary subjection to hydrolysis followed by neutralization: disaccharides (sucrose, trehalose); trisaccharides (raffinose, melizitose); polysaccharides (dextrin, starch, inulin, glycogen, cellulose); glucosides (aesculin, amygdalin, arbutin, phloridzin, strophantin, picrotoxin, salicin, saponin); gums (arabic, acacia, tragacanth); glycoproteins (mucin, ovomucoid); glycolipins (phrenosin); nucleic acid.

Compounds other than carbohydrates also reduced. Cysteine gave a green color; lactic acid, a royal blue; oxalic acid, a green; mucic acid and citric acid, dark blue; tartaric acid, a royal blue with a purplish tinge; and pyruvic acid, green.

The reagent, however, is more sensitive to the sugars than to the organic acids. The limit of sensitivity for glucose is 0.03%. Citric acid reduces in a 3% solution, but not in a 2% solution. Tartaric acid reduces in a 2% solution, but not in a 1% solution. Lactic acid does not react in a 0.9% solution. Oxalic acid gives a faint reaction in a 4% solution, but no reaction in a 3% solution. Pyruvic acid gives a green color in a 5% solution, but not in a 4% solution. Mucic acid proves positive in a 3% solution, but not in a 2.5% solution. Cysteine hydrochloride gives a green color in a 0.5% solution, but no color in a 0.25% solution.

Chloroform, uric acid and allantoin, creatinine, formaldehyde, acetaldehyde, acetone and β-hydroxybutyric acid do not respond. Ethyl acetoacetate reacts in the negative, but after hydrolysis with dilute hydrochloric acid it gives a faint green color. Proteins do not interfere with the reaction.

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Oxygen Consumption of the Cardiac Ganglion of Limulus Polyphemus.*

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(Introduced by W. H. Chambers.)

From the Marine Biological Laboratory, Woods Hole, Mass.

The CO_2 production of the cardiac ganglion of Limulus has been studied by Garrey,[1] who found a direct quantitative relation between the rate of the heart beat and the rate of CO_2 formation in the ganglion. Both of these rates were augmented when the ganglion was stimulated chemically, mechanically, or electrically, and diminished when the inhibitory nerves were stimulated.

The object of our experiments was to study the oxygen consumption of the ganglion during rest and during electrical stimulation.

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* This work was performed under the direction of Dr. W. E. Garrey and Dr. R. W. Gerard as a part of the Physiology Course of 1930 at the Marine Biological Laboratory, Woods Hole, Mass. We wish to acknowledge our indebtedness to them for their advice and help and for permission to publish our results.

[1] Garrey, W. E., *J. Gen. Physiol.*, 1920, **3**, 163; 1921, **4**, 149.

The method employed was the Warburg manometric technique.[2] For stimulation experiments we used chambers with simple manometers and platinum electrodes sealed in, as employed by Gerard.[3] The ganglia were kept in sea water instead of Ringer's solution. At least 5 ganglia, having a combined moist weight of about 0.1 gm., were used. After careful dissection they were kept cold until mounted on the electrodes, after which the experiment was conducted at a temperature of 24.6 to 24.8°C.

Readings were taken every 10 or 15 minutes for a period of about 3½ hours. After a resting period of one-half to one hour, stimulation was applied for 10 minutes at the rate of 2 shocks (one make and one break) per second by means of a Harvard inductorium with coil set at about 4 cm. and one dry cell. Another resting period of about 40 minutes was allowed to elapse before the next stimulation was given, and this procedure was repeated several times. At the end of the experiment the dry weight of the ganglia was obtained, and from this the moist weight was calculated on the basis of 80% water.

The table shows results obtained in 3 experiments when stimulation was given. The average resting value for these 3 experiments was 104 cu. mm. of oxygen per gm. of moist weight per hour, with variations between 93 and 120 cu. mm., while the average obtained in 9 experiments was 114 cu. mm., with variations between 30 and 217. Precautions as to condition and number of ganglia used were observed with especial care in the experiments of July 25, 28, and 29.

The depression of oxygen consumption which accompanied elec-

TABLE I.

Oxygen Consumption of Ganglia per Hour.

Cu. mm. per gm. moist weight.

	July 25*	July 28*	July 29†
Rest	117	97	99
Stimulation	34	24	0
Rest	107	103	110
Stimulation	26	11	94
Rest	127	93	93
Stimulation	0		63
Rest	111		98
Stimulation			44
Rest			120
Stimulation			50
Rest			116

* Stimulation of posterior ends of ganglia.

† Stimulation of anterior ends of ganglia.

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[2] Warburg, O., *Über den Stoffwechsel der Tumoren*, Berlin, 1926.

[3] Gerard, R. W., *Am. J. Physiol.*, 1927, **82**, 381.

trical stimulation of the posterior ends of the ganglia was very striking in its magnitude and consistency of appearance. The average depression amounted to over 80% of the resting oxygen intake. A similar effect, but in a lesser degree, was observed when shocks were applied to the anterior ends of the ganglia. There was a prompt return to the resting rate when stimulation ceased.

Stimulation, at the rate of 2 shocks per second, of the ganglion of an isolated Limulus heart preparation was found by Asher and Garrey[4] to produce definite depression of the heart rate, and this was confirmed by Garrey and Knowlton,[5] who also found that stimulation at a faster rate produced complete inhibition of the entire ganglion. Therefore it is probable that the fall in oxygen consumption which we observed was due to inhibition. This is in harmony with Garrey's conclusion[1] that inhibition involves a decrease in cellular metabolism.

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A Life Cycle of a Thermophilic Organism.

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Numerous investigators have described organisms capable of growing at 62°C. This is the accepted pasteurization temperature for milk. In all of the descriptions of such thermophilic phenomena, especially where milk is concerned, one characteristic has been quite apparent—that is, sharp rather than gradual increases in bacterial numbers. Our observations have been in accord with those described in the literature. Calculation of the generation time for these increases has disclosed values of 5 to 8 minutes. It was thought that such short generation times were in little accord with accepted views. The only alternative was to consider the phenomena as evidence of something other than growth. Spores are usually present at the same time vegetative cells appear in smears of milk. It has been assumed that these spores are germinating rather than that the vegetative cells are sporulating. If spores exist in milk which shows thermophilic growth they must be present in large numbers.

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[4] Asher, L., and Garrey, W. E., *Am. J. Physiol.*, 1930, **94**, 619.

[5] Garrey, W. E., and Knowlton, F. P., (personal communication).

In our experiments, milk diluted to 1 part to 100,000,000 disclosed micro-organisms in the very highest dilutions. The protocol of such an experiment is given in Table I. It will be noted that there is a change of flora between the fourth dilution and the seventh, leaving a gap of 2 dilutions. This gap is rather characteristic of our experiments.

TABLE I.
Showing effect of diluting milk containing thermophiles to dilutions of 100,000,000. Note absence of growth in dilutions 5 and 6.

Temp.	Plate	1	2	3	4	5	6	7	8
30	1300	+	+	+	+ cocci	—	—	+ rods	—
37	800	+	+	+	+ cocci	—	—	—	+ rods
60	0	+ rods	+ rods	—	—	—	—	—	—
Transfers	+	+	+	+ rods	—	—	+ rods		

Hourly direct counts 0 up to 11 hours. 10,000,000—12 hour. Generation time 7.5 minutes.

Continuous observations of this character have led us to the belief that there is a definite life cycle in the organism which we have been studying. It would be interesting if similar cycles were shown to exist for other organisms. Briefly, the cycle is as follows:

1. An invisible spore; present in large numbers, heat resistant, incapable of growth on ordinary laboratory media either at 37°C. or at 60°C.
2. A vegetative cell resulting from germination of (1); easily destroyed by heat, capable of growth upon ordinary laboratory media.
3. A spore forming within the rod (2); heat resistant.
4. A spore (3); devoid of all stainable material, heat resistant, incapable of growth on ordinary laboratory media.
5. A spore; invisible, heat resistant, not capable of growth upon ordinary laboratory media, dormant. This dormancy is broken by heat, cold, chemicals, such as alkali and acids.

Changing the organism from stage 5 to stage 1 makes it capable of germination, thus starting the cycle over again. A modification of this theory would permit the sequence of events to be stage 1, stage 3, stage 2, and so on. In our experiences such a theory more nearly explains many of the anomalous results reported in the literature, especially where thermophilic organisms in milk are concerned.

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Thyroid Hyperplasia Produced in Chickens by Ultraviolet Light Deficiency.

KENNETH B. TURNER. (Introduced by W. W. Palmer.)

From the Department of Medicine of the College of Physicians and Surgeons, Columbia University, and the Presbyterian Hospital, New York City.

A group of 4-week old Barred Rock chickens was divided into two lots. Both lots of chickens received the same diet with the addition of cod liver oil from time to time. Both lots were raised indoors. The first lot of chickens which was used as a control received light through ordinary window glass supplemented by ultraviolet radiation twice a week by means of a mercury vapor lamp. The second lot of chickens was raised under No. 48 Pittsburgh amber glass which has been shown to exclude effectively the ultraviolet rays.

About 70 chickens of which half were controls have been used in this experiment. The chickens in both groups were vigorous, well-nourished, and well-developed. There was no essential difference in plumage. There was little or no evidence of parathyroid enlargement. The chief finding in the chickens raised under amber glass was an enlargement of the thyroid glands. This is well shown by Table I which gives the results on the latest group of 31 birds.

TABLE I.

	Age	Avg. body weight	Weight of thyroids in mgm.		
	days	gm.	min.	max.	avg.
8 controls	62-109	430	16	34	27
8 ultraviolet deficient	” ”	425	28	100	48
7 controls	122-164	1021	34	74	54
8 ultraviolet deficient	” ”	1010	40	226	124

In addition to the increase in size of the thyroids of the chickens deprived of ultraviolet light, these glands were of a deep purplish red color as opposed to the light pink of the normal gland. Microscopically the enlarged glands showed evidence of marked epithelial hyperplasia with striking disappearance of colloid.

It would seem from this experiment that ultraviolet light deprivation is a powerful goitrogenic agent in Barred Rock chicks.

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Determination of Serum Proteins.

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There have been two methods for the estimation of the serum proteins, the refractometric and the chemical determination of the nitrogen through some modification of the Kjeldahl method. While titrating proteins electrometrically we found that the amount of acid required within certain ranges to take a protein from one pH to another was a direct function of the concentration of the protein actually present. This offered an ideal and simple method for the estimation of the serum proteins in the normal and pathological blood. For clinical purposes this can be done with indicators of which we have found methyl red the most satisfactory, and for more exact estimations, the hydrogen ion concentration can be determined electrometrically.

For the colorimetric estimation of the proteins in the serum the following method is used. One-half cc. of blood is diluted with an equal quantity of distilled water and one-quarter cc. of tenth normal HCl and 2 drops of methyl red are added. A color develops instantly.

Yellow—over 6% Protein.
Yellow orange—5% Protein.
Pink orange—4% Protein.
Pink—3% Protein.
Red—below 3% Protein.

This furnishes results sufficiently accurate for ordinary clinical purposes. If more accurate results are required a set of acetate buffers are made up between the ranges of pH = 4 and pH = 6. The serum proteins may then be matched as follows:

7.2%----pH 6.2
6.3%----pH 5.9
5.4%----pH 5.6
4.5%----pH 5.3
3.6%----pH 5.0
2.7%----pH 4.7

The application of protein titration given above is entirely designed for clinical purposes. The results when compared with buffer solutions give results that check within 0.4-0.5 gm. of protein per 100 cc. of serum with those obtained by the determination of the total protein nitrogen by digestion methods. This is sufficiently accurate for diagnostic purposes. One hundred sera were examined and parallel determinations of the serum protein nitrogen made and were found to be within this limit. In 3 cases of nephrosis with edema a distinctly red color was obtained and the protein digestion method showed contents of 3.1, 2.9 and 3.4 gm. per 100 cc. respectively. The turbidity of the sera due to the high lipoid content did not interfere. One case of suspected nephrosis with slight edema showed an orange red color equivalent to a buffer of pH 5.2 and was shown by the standard methods to have 4.5% of protein. Certain of the sera which gave a yellow orange color (pH = 5.8) contained about 6% total protein and were to be classified as low normals. In cases of cachexia of cancer an orange pink color (pH = 5.3) was obtained and the sera showed a protein content of 4.6% and 4.8% respectively.

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Concentration of the Causative Agent in the Filtrate of the Rous Chicken Sarcoma.

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The studies of Lewis and Andervont,[1] and those of Michaelis,[2] also of Baker and MacIntosh,[3] Frankel[4] and Sugiura and Benedict,[5] within the past 2 or 3 years, have disclosed additional information concerning the physical and chemical properties of the causative agent of the Rous Chicken Sarcoma. Most of these observers investigated the range of H ion concentration in which the active agent retains its infectivity, and concluded that the nature of the buffer solution appears to be of no great significance, as long as the pH is between 3.8 and 10 to 11. Since the experiments of Lewis and Michaelis were carried out with tumor extracts, and those of Sugiura and Benedict with fragments of tumor tissue, we were interested in determining the activity of the causative agent in a cell-free filtrate under varying pH values, and to correlate this with the results obtained with the tumor extract, or tissue fragments.

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[1] Lewis and Andervont, *Bull. Johns Hopkins Hosp.*, 1927, **41**, 185.

[2] Lewis and Michaelis, *Bull. Johns Hopkins Hosp.*, 1928, **43**, 92.

[3] Baker and MacIntosh, *Brit. J. Exp. Path.*, 1927, **8**, 257.

[4] Frankel, E., *Z. f. Krebsforschung*, 1929, **29**, 491.

[5] Sugiura and Benedict, *J. Can. Res.*, 1927, **11**, 164.

We found that buffering the active filtrate with a potassium hydrogen pthalate solution to pH 4 caused the solution to become cloudy, with subsequent precipitation upon standing. Upon centrifuging the suspension for about 30 minutes, a heavy precipitate was formed, and the supernatant fluid became clear. The clear supernatant fluid when tested by inoculation into chickens, is non-infective. Only when it remains slightly cloudy, indicating that all of the protein substances have not been carried down in the precipitate, will an occasional tumor result after a much delayed period of time. The precipitated portion while still at pH 4 when injected into chickens is only slightly infective, that is, its infectivity seems somewhat reduced. In other words, the active agent is carried down in the precipitated portion, and rendered partly inactive at this H ion concentration.

When the supernatant fluid is decanted, and the remaining precipitated residue is washed several times with a buffer solution at pH 4, and then resuspended in a Clarke's standard buffer solution adjusted to pH 8, the causative agent is liberated, and the supernatant fluid becomes so highly infective that doses of 0.2 cc. will cause a tumor in from 8 to 11 days. The residue from this washing at pH 8 is also slightly potent.

The potency of the causative agent in the supernatant fluid at pH 8 can again be inhibited by bringing the solution to pH 4, for at this degree of acidity, a precipitate again forms, which contains the active principle, and the supernatant fluid becomes inactive. The non-potency of the supernatant fluid at pH 4 is not due to the acidity for when neutralized, it still fails to produce a tumor.

After 3 or 4 re-precipitations of the agent at pH 4, the bulk of the final precipitate may be reduced to 1 to 2 mg., dry weight, whereas the first precipitate might weigh 30 mg. The infectivity of this concentrated precipitate after it has been extracted by a buffer solution at pH 8 equal in volume to the original filtrate, is not only fully retained, but even seems to be enhanced. For example, where it required 0.5 cc. of the original filtrate to obtain a tumor, the concentrated precipitate suspended in the same volume of fluid at pH 8, gave rise to tumors in doses of 0.2 cc. At times, the washings from the first precipitate gave tumors with 0.1 cc. The agent is evidently more virulent in the partially purified form than in the original filtrate, as it killed the chicks in about 20 days, whereas the length of life of the chicks after inoculation with the original filtrate averaged 28 days.

The moist concentrated precipitate will maintain its infectivity when kept on ice for 4 to 5 days. Moreover, when dried in the desiccator *in vacuo,* and kept for 10 days or longer, it is still potent, though its infectivity is considerably lessened. As a parallel experiment, the filtrate itself was dried in the desiccator and found to be non-potent after 24 hours.